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Effects of Atrazine on 'Zostera marina' in Chesapeake Bay, Virginia

Virginia Inst. of Marine Science
Gloucester Point

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This project was designed to assess the effects of agricultural herbicides on submerged aquatic vegetation in the Lower Chesapeake Bay. Atrazine was selected for testing because it is the most widely utilized herbicide in the Bay region. Zostera marina was the submerged vegetation studied.

This project began with two surveys. The first survey, conducted in 1979, covered forty eight stations throughout the Virginia portion of the Chesapeake Bay. The survey was designed to indicate typical atrazine loading for the lower Bay. A second survey in 1980 was limited to the Severn River, and was intended to indicate the duration of peak atrazine loading. Information generated in the two surveys was utilized to design dosing experiments.

Field dosing experiments, utilizing large plexiglass enclosures, measured effects of short-term atrazine exposure on the net production of the Zostera community. Greenhouse experiments, utilizing a flow-through dosing system, measured effects of long-term (21 day) atrazine exposure on the morphology of mature Zostera plants.

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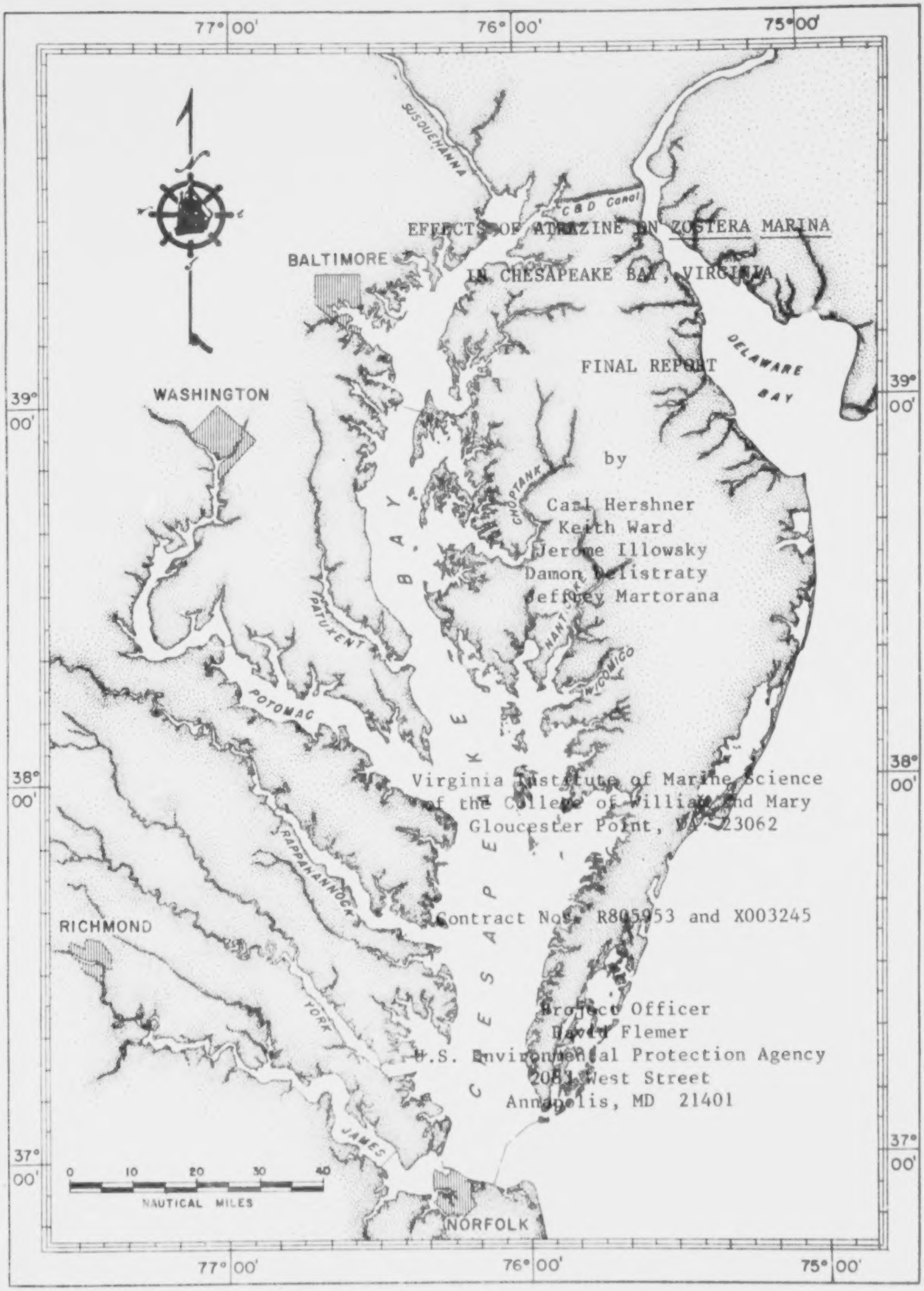
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IN CHESAPEAKE BAY, VIRGINIA

FINAL REPORT

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NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

This project was designed to assess the effects of agricultural herbicides on submerged aquatic vegetation in the lower Chesapeake Bay. Atrazine was selected for testing because it is the most widely utilized herbicide in the Bay region. Zostera marina was the submerged vegetation studied.

The project began with two surveys. The first survey, conducted in 1979, covered forty eight stations throughout the Virginia portion of the Chesapeake Bay. The survey was designed to indicate typical atrazine loading for the lower Bay. A second survey in 1980 was limited to the Severn River, and was intended to indicate the duration of peak atrazine loading. Information generated in the two surveys was utilized to design dosing experiments.

Field dosing experiments, utilizing large plexiglass enclosures, measured effects of short-term atrazine exposure on the net production of the Zostera community. Greenhouse experiments, utilizing a flow-through dosing system, measured effects of long-term (21 day) atrazine exposure on the morphology of mature Zostera plants.

Adenine nucleotide concentrations and a ratio of those concentrations (termed adenylate energy charge) was assessed in Zostera exposed to various concentrations of atrazine in laboratory dosing studies. Adenylate determinations proved a more sensitive indication of stress than either the oxygen production measurements or the morphometric determinations utilized in the field and greenhouse studies. In combination with those studies, the adenylate studies provide evidence for a resistance in Zostera to low (less than 10 ppb) levels of atrazine, and short term adaptation to atrazine concentration around 100 ppb.

The entire series of investigations is concluded to indicate that atrazine effects on mature Zostera marina plants are probably not the principal cause for the recent decline in distribution of eelgrass in the lower Chesapeake Bay. Several limitations of the study and suggestions for future work are included.

This report was submitted in fulfillment of Contracts R805953 and X003245 by the Virginia Institute of Marine Science under the sponsorship of the U. S. Environmental Protection Agency and the Ciba-Geigy Corporation. This report covers the period September 1, 1978 to August 31, 1982.

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SECTION 1

PROJECT OVERVIEW

INTRODUCTION

The decline of submerged aquatic vegetation in the Chesapeake Bay during the 1970's led to much speculation about potential causes. Among the factors considered were agricultural herbicides. The initial hypothesis was that increased levels of herbicides were being carried into the Bay by storm runoff producing concentrations sufficient to kill the submerged vegetation. Preliminary literature reviews and land use studies indicated that herbicide use was increasing in the Bay watershed, thus providing at least circumstantial evidence for the hypothesis. The project reported here was designed to specifically investigate the hypothesis and produce evidence of the degree to which agricultural herbicides were affecting submerged aquatic vegetation.

RESEARCH APPROACH

Atrazine has been used throughout this investigation as our model herbicide. It was selected because it is the herbicide utilized in the largest quantity within the lower Chesapeake Bay watershed. Atrazine is a triazine herbicide whose principal mode of action is disruption of the Hill reaction in photosynthesis. Its principal application is for control of weeds in cornfields. The herbicide is typically applied as a preemergent spray to fields in the spring of the year. It has found increasing use with the spread of no-till planting methods and is sometimes applied in combination with other agricultural chemicals.

Zostera marina is the species of submerged aquatic vegetation which has been studied. It is the predominate subtidal vegetation of the lower Chesapeake Bay and because of the recent declines in distribution has been the focus of other studies in Virginia undertaken as part of the EPA's Chesapeake Bay Program.

The research approach utilized in this study was to first determine the level of Zostera marina's exposure to atrazine, and then test for effects caused by that level of exposure. To that end, this project has been divided into two general lines of investigation. The first effort was a survey program to monitor levels of atrazine in water and sediments in the lower Bay. Forty eight sites were sampled four times during 1979. The samplings were generally timed to occur before and immediately after spring application of herbicides and before and after fall harvesting. This schedule was intended to allow detection of peak seasonal loadings of atrazine in Bay waters. In 1980 a second survey was conducted in the Severn River. This survey was designed to gather information about the duration of the peak spring loadings identified by the first survey program. Together these survey programs provided information on the *in situ* levels of atrazine experienced by *Zostera marina*.

The second line of research was a variety of experiments designed to identify effects of atrazine on *Zostera*. Using the information collected in the survey programs, we selected a range of atrazine concentrations and two general exposure periods for testing. The selections were made to ensure that we tested both typical and extreme conditions.

We selected three test parameters in the investigation for effects. Oxygen production was monitored during short term *in situ* exposures of the entire *Zostera* community. Aboveground morphometrics were monitored during long term laboratory exposure of individual *Zostera* plants. Finally adenylate energy charge was monitored during both short and long term laboratory exposures.

CONCLUSIONS

The survey of Virginia waters of the Chesapeake Bay indicated that *Zostera marina* in either its current or recent distributions has generally not been exposed to levels of atrazine in excess of 1 ppb. Using the Severn River as a model system, a survey program suggested that even in "worst case" situations, the exposure of *Zostera* to elevated levels of atrazine (in excess of 1 ppb) was short term (one week or less). With this information in mind field and laboratory dosing experiments were undertaken to test the effects of naturally occurring atrazine concentrations on *Zostera*. Field studies indicated that *Zostera* productivity, as measured by oxygen production, is consistently depressed by atrazine concentrations of 1000 ppb. Concentrations of 100 ppb frequently caused depression of productivity but results at this and lesser concentrations were so variable as to prevent statistically significant conclusions. Field experiments designed to test effects of simultaneous exposure to atrazine and reduced light produced no evidence of either additive or synergistic effects.

Long-term exposure (21 days) of *Zostera* to atrazine in greenhouse experiments demonstrated that atrazine could produce significant effects on *Zostera* morphology at concentrations greater than 60 ppb. The morphometric test parameters proved so variable and the range of concentrations tested was so wide that no statistically significant conclusions could be drawn.

Analysis of adenine nucleotide concentrations in *Zostera* tissues proved to be a potentially sensitive indicator of stress. In short term exposures (six hour), adenylate concentrations were altered by atrazine concentrations of both 10 ppb and 100 ppb. In long-term exposures (twenty one days) sublethal stress caused by exposure to atrazine concentrations of 0.1 ppb, 1.0 ppb and 10 ppb was indicated by a change in the ratio of adenylate concentrations (termed "energy charge"). Summarization of the adenylate experiments suggested that mature *Zostera* is able to withstand exposure to low levels of atrazine (10 ppb and less) for periods in excess of 21 days. Exposure to higher levels of atrazine (100 ppb and 1000 ppb were tested) apparently elicits physiological changes which can support the plant for only shorter periods of time.

From all of these investigations we are led to believe that the effects of atrazine on mature *Zostera marina* are probably not a major causative factor for the recent declines in distribution within the Virginia portion of the Chesapeake Bay. Our work indicates that while atrazine can produce lethal and sublethal effects on *Zostera*, the herbicide is not found in areas presently or formerly inhabited by *Zostera* at concentrations high enough or persistent enough to exceed the plant's ability to resist the imposed stress. This conclusion must be considered in light of several limitations of these investigations. First, we have only addressed effects on mature plants. No work is reported here on reproduction, germination or seedling growth. Second, we have only addressed the effects of atrazine as a sole stressor. No work was undertaken to evaluate additive or synergistic effects with other chemicals. The investigation of light and atrazine interaction was inconclusive and light was the only environmental parameter addressed.

Third, all of the work conducted here was undertaken with *Zostera* plants and their naturally occurring epiphyte community. For purposes of this study, which was designed to address potential management questions, analysis of effects on the natural assemblage was appropriate. However, interpretation of results of the dosing studies must be cognizant of the lack of any data partitioning effects among the assemblage's components. A fourth consideration is that the work reported here is focused on dissolved atrazine which we believed to be the principal mode of exposure for *Zostera*. We have not analyzed the impacts of atrazine sorbed to suspended sediments which may also be a significant mode of exposure. A final consideration is the lack of any quantitative data assessing the condition of *Zostera* returned to control conditions after exposure to atrazine.

With all of the limitations of this investigation in mind, we believe our data suggest management or regulation of agricultural herbicide usage will not prove a panacea for the decline of *Zostera marina* in the Chesapeake Bay.

RECOMMENDATIONS

The limitations of this investigation suggest several avenues for additional work. The effects of atrazine on *Zostera* germination and seedling growth remain a significant question. Synergistic effects of atrazine with other chemical and physical stressors also remains an interesting question. It is apparent from this work that detection of herbicide impacts on Zostera requires fairly sensitive analytical methods. The sublethal effects potentially caused by typical herbicide loadings are of a magnitude which is not especially amenable to analysis by the morphometric or production measurements used in some of our studies. If those types of analyses are attempted, our experience indicates efforts must be made to obtain large numbers of replicates and special attention must be given to collection of ancillary data which can be used to factor out response variations due to the composition of the natural community.

SECTION 2

ANALYTICAL METHODS

INTRODUCTION

The methods utilized for analysis of atrazine in all of the succeeding work in this report are based on standard chromatographic procedures. Procedures were modified as detailed below after consultation with personnel working in the Ciba Geigy Corporation laboratories in Greensboro, North Carolina.

All of the analytical work was conducted in a small laboratory set up exclusively for this project. A rigorous quality assurance program was undertaken to ensure the accuracy of the atrazine concentrations reported.

WATER

Estuarine water was collected in amber glass bottles with teflon lined caps and stored under refrigeration until analysis. Subsamples were filtered through Reeve-Angel 802 and Whatman 2V filter papers. Powdered sodium sulfate (approximately 3-5 gm) was dissolved in the water in an effort to reduce possible emulsions. All water samples were extracted with methylene chloride (3 x 50 ml) which was then passed through anhydrous granular sodium sulfate and reduced in volume by rotary evaporation to approximately 1 ml. Extracts were quantitatively transferred to graduated centrifuge tubes with methylene chloride, evaporated just to dryness under nitrogen, and volumes adjusted with toluene. Most water extracts were sufficiently clean for direct GC analysis. The minimum detection limit (MDL) for atrazine in water was set at 0.10 ppb.

SEDIMENT

The procedure used is modified from Mattson et al., 1970 on the basis of discussions with Ciba Geigy Corporation personnel in Greensboro, North Carolina.

Homogenized sediment samples (100 gm wet) were refluxed one hour in water/acetonitrite (1:10, 300 ml). The resulting extract was filtered through Reeve-Angel 802 and Whatman 2V filter papers. Using the water content determination from a dried subsample and the volume of recovered extract, the dry-weight equivalent of sediment was calculated. The extract was placed on a steam bath under nitrogen, reduced in volume to approximately 100 ml, transferred to a separatory funnel, and diluted to one liter with water. Powdered sodium sulfate (approximately 5 gm) was dissolved in the water to reduce emulsions. The extract was then partitioned against methylene chloride (3 x 50 ml) which was then passed through anhydrous granular sodium sulfate and rotary evaporated just to dryness. The residue was quantitatively tranferred to an alumina column (25 gm Grade V; Kontes K-420 280, 22 mm o.d.) in carbon tetrachloride (10 ml). The column was rinsed with an additional 20 ml carbon tetrachloride which was discarded. The column was eluted with carbon tetrachloride (80 ml) and then ethyl ether/carbon tetrachloride (1:20, 100 ml). The eluate was rotary evaporated just to dryness and quantitatively transferred to graduated centrifuge tubes with methylene chloride. The extract was concentrated to dryness under nitrogen and volume adjusted with toluene. The MDL for atrazine in sediment was set at 5.0 ppb.

GAS CHROMATOGRAPHIC PARAMETERS

Analysis of water and sediment extracts were performed using a Tracor 560 gas chromatograph equipped with a model 702 nitrogen-phosphorus detector under the following parameters:

Column: 3% Carbowax 20 M 80/100 Chromasorb WHP
(well conditioned) 4' x 2 mm i.d. glass

Temps: Oven, 210°C; injection port, 230°C;
detector, 275°

Flows: (carrier) He 40 ml/min, ultra high purity
(plasma gases) H_2 3.0 ml/min, ultra high purity
Air set at 40 psi at regulator; zero grade

NP source power: 810, background set at 75% FSD at 1 x 4
attenuation with zero off

Chart speed: 0.25"/min

Linearity plots were made with each GC run. Standards within 10% of the atrazine value in environmental extracts were injected after all positive samples. Calculations were based on the analytical standard immediately after each positive sample.

QUALITY ASSURANCE PROGRAM

A rigorous internal and external laboratory and analytical quality assurance program was maintained throughout the course of the project.

Internal laboratory and analytical quality assurance included the following.

1. Stock atrazine standards were usually prepared every three months; working standards were prepared each month. Stock standards were stored in a freezer. Working standards and sample extracts were refrigerated between gas chromatographic analyses.

2. A linearity plot of standards, covering the range and attenuation at which the extracts were analyzed, was developed at the beginning of each GC run. A standard within 10% of the sample peak height was injected immediately after each positive sample.

3. Sample blanks and samples fortified over a range of atrazine concentrations were carried through the analyses periodically to assure consistency in recovery and reproducibility.

4. A limited access laboratory was maintained. The lab, lab instruments, and glassware were used only for atrazine determinations. Only personnel involved directly with the atrazine determinations were permitted access.

5. All glassware used for these analyses was detergent-washed, rinsed with tap water, distilled-deionized water, acetone, toluene, and hexane.

6. All reagents and supplies to come in contact with the samples, such as glass wool, sodium sulfate, XAD-2 resin, cellulose extraction thimbles, teflon boiling chips, etc. were exhaustively extracted by Soxhlet in acetone, toluene, and hexane, or, in some cases, methylene chloride.

7. Solvents were checked for purity periodically by concentration of 500 ml to 1 ml for subsequent analysis by GC. (Burdick and Jackson glass distilled solvents were used). Distilled-deionized water was checked for contamination by extraction of one liter and GC determinations. Ultra-high purity hydrogen and helium, and zero grade air were used as GC gases; high purity nitrogen was employed for concentration of small volumes of extracts (Linde/Union Carbide specialty gases).

8. Water samples were collected in amber glass bottles with teflon lined caps and refrigerated in the dark until analysis. Sediment and vegetation samples were collected in glass jars with aluminum foil-lined caps or equivalent containers and frozen until analysis.

9. All chromatograms were labeled, dated, and stored for raw data retrieval. Standard lab sheets were maintained for documentation of sample number, substrate, station, dates of collection, extraction, and analysis, volumes of sample extracted and injected, as well as peak heights of samples and standards.

10. Samples were extracted as soon as possible after collection, however, in some cases several months elapsed before lab workup took place.

External quality assurance consisted of analyses of "blind" or unknown QA samples submitted by outside agencies such as EPA Annapolis Field Office and Research Triangle Institute, Research Triangle Park, N.C. Each of these agencies also conducted on-site evaluations of the VIMS herbicide laboratory. Those performance evaluations involved fortification of estuarine water at levels of atrazine between 0.21 ppb and 65.5 ppb. The VIMS recoveries were usually well within 10% of the true values (Gaskill and Jayanty, 1981).

REFERENCES

Gaskill, A., Jr. and R. K. M. Jayanty. 1981. Second performance audit of the VIMS herbicide monitoring program. Report submitted to Environmental Protection Agency, Research Triangle Park, North Carolina. 25 pp.

Mattson, A. M., R. A. Kahrs, and R. T. Murphy. 1970. Quantitative determination of triazine herbicides in soils by chemical analysis. Residue Reviews 32:371-390.

SECTION 3

1979 LOWER CHESAPEAKE BAY SURVEY PROGRAM

INTRODUCTION

The 1979 survey of the lower Chesapeake Bay was designed to identify the levels of atrazine in water and sediments during one growing season. A preliminary assumption was that atrazine levels would fluctuate seasonally, reaching maxima immediately after field applications in the spring and, perhaps again, following harvesting in the fall. The survey was therefore designed to sample a large number of sites four times during the year, with timing selected to correspond to spring and fall farming activities. The results of the survey were intended to establish the actual range of atrazine concentrations to which *Zostera marina* might be exposed.

METHODS

Forty-eight sampling stations in the lower Chesapeake Bay were identified and occupied four times during 1979. Stations were selected primarily to provide a wide coverage of the Bay shoreline and major tributaries. Specific site selection was governed principally by available access. Wherever possible, however, sites which either have or had *Zostera* beds were selected. Sample collection was scheduled so that the first sampling round occurred prior to any farming activities in the spring. The second round occurred immediately after the first major rainstorm following spring application of herbicides to the fields. The third round was generally late summer and prior to the fall harvesting of crops. The fourth round was conducted after most fields were harvested.

Samples were collected either from a small boat or by wading to the nearshore site. Sub-surface water was collected in solvent rinsed, amber glass bottles with teflon lined lids. Sediments were collected with an 18 cm^2 coring tube. Several cores were taken at each station and the top 5 cm of each core was collected and stored in either glass jars with aluminum foil lined caps or equivalent containers. Water samples were refrigerated and sediments were frozen until analysis.

See the analytical methods section (Section 2) of this report for sample analysis techniques.

RESULTS

The survey stations are listed in the appendix to this section, Table A3.1. The sites are located on a general area map, Figure 3.1 and on river

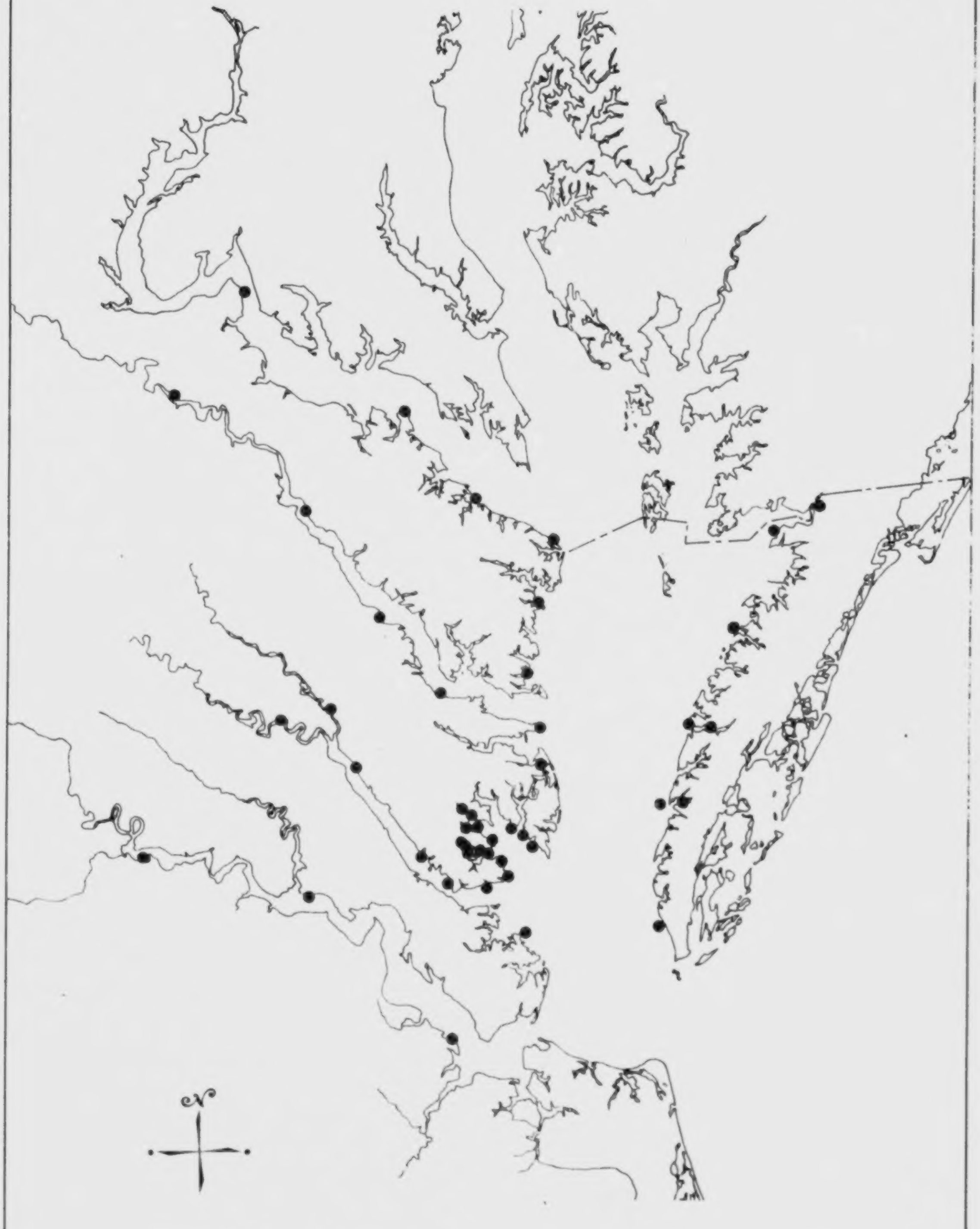


Figure 3.1 - Locations of Lower Chesapeake Bay Survey Stations

system maps included in the appendix, Figure A3.1 through Figure A3.15. The concentrations of atrazine in water samples are reported in Table 3.1. The concentration of atrazine in selected sediments samples are reported in Table 3.2.

DISCUSSION

All discussion of Zostera marina distributions are based on Orth et al., 1979.

The four Chesapeake Bay stations are allocated in areas which have or had Zostera beds. Zostera was not found in any of the samples collected for this survey, but it exists in significant quantities in all the areas except the Cricket Hill/Gwynn's Island site. In that area, Zostera is still found in small beds at the entrances to Milford Haven. Water samples collected at the Chesapeake Bay stations never contained detectable amounts of atrazine.

None of the James River sites have any Zostera, nor have they had any in the recent past. Atrazine was detected in water samples collected after spring field applications. Concentrations were generally highest at the upriver sites, but no sample ever exceeded a 1 ppb level.

In the York River system, sites at the head of the river do not now have, nor previously had, submerged aquatic vegetation. The sites at Carter Creek and Mumfort Island, in the middle of the system, formerly had extensive grass beds, but neither site supports Zostera presently. The sites at the mouth of the river all have extensive grass beds currently. Water samples from the York system contained detectable amounts of atrazine only in the post-application survey round and the later summer survey round. At those times the concentrations were detectable only at sites above Carter Creek. None of the detectable concentrations exceeded 1 ppb.

The Severn River system was the most intensively sampled system in this survey. As in the York River, Zostera is only found near the river mouth at the Turtle Neck Point station. Zostera may have previously occurred at the next two stations upriver, School Neck Point and Cod Point, but there are no records of any further upriver extensions. Analysis of water samples always detected atrazine at the station in the headwaters. The result is not unexpected since the station is located in the principal drainage channel for much of the agricultural land in the drainage basin. Atrazine concentrations in water samples exceeded 1 ppb only in the second survey round, completed immediately after spring field applications. Concentrations were regularly near or below the detection limits at the three downstream stations. Concentrations never exceeded the detection limits at the river mouth where the grass beds are currently found.

The Ware River system has extensive grass beds at the three down-river stations. These beds have apparently been relatively stable through the recent past. The upstream stations do not now have, and may never have had, any grass beds. Atrazine was detected in water samples at all stations following the spring field applications. At that time concentrations were relatively uniform along the length of the river, with a maximum concentration

TABLE 3.1. CONCENTRATIONS OF ATRAZINE IN WATER SAMPLES FROM THE LOWER CHESAPEAKE BAY DURING 1979
(all concentrations in part per billion)

	Circuit #1		Circuit #2		Circuit #3		Circuit #4	
Station	Date	Atrazine	Date	Atrazine	Date	Atrazine	Date	Atrazine
CB1	4-14-79	≤0.10	6-8-79	≤0.10	8-10-79	≤0.10	12-4-79	≤0.10
CB2	4-14-79	≤0.10	6-8-79	≤0.10	8-10-79	≤0.10	12-4-79	≤0.10
CB3	4-14-79	≤0.10	6-8-79	≤0.10	8-10-79	≤0.10	12-4-79	≤0.10
CB4	4-14-79	≤0.10	6-8-79	≤0.10	8-10-79	≤0.10	12-4-79	≤0.10
JR1	4-25-79	≤0.10	6-5-79	0.20	8-7-79	0.19	12-5-79	0.50
JR2	4-25-79	≤0.10	6-5-79	0.15	8-7-79	0.19	12-5-79	≤0.10
JR3	4-25-79	≤0.10	6-5-79	≤0.10	8-7-79	0.19	12-5-79	≤0.10
YROA	3-22-79	≤0.10	5-29-79	≤0.10	8-8-79	0.26	11-28-79	≤0.10
YROB	3-22-79	≤0.10	5-29-79	0.22	8-8-79	(b)	11-28-79	≤0.10
YROC	3-22-79	≤0.10	5-29-79	≤0.10	8-8-79	0.20	11-28-79	≤0.10
YR1	3-22-79	≤0.10	5-29-79	0.20	8-8-79	0.12	11-7-79	≤0.10
YR2	3-22-79	≤0.10	5-29-79	≤0.10	8-8-79	≤0.10	11-7-79	≤0.10
YR3	3-22-79	≤0.10	5-29-79	≤0.10	8-8-79	≤0.10	11-7-79	≤0.10
YR4	3-22-79	≤0.10	5-29-79	≤0.10	8-8-79	≤0.10	11-7-79	≤0.10
YR5	3-22-79	≤0.10	5-29-79	≤0.10	8-8-79	≤0.10	11-7-79	≤0.10
SR1	4-3-79	0.33	5-14-79	108.11	6-25-79	0.27	11-15-79	0.30
SR1B	4-3-79	(1)	5-14-79	19.89	6-25-79	0.21	11-15-79	≤0.10
SR2	4-3-79	≤0.10	5-14-79	4.02	6-25-79	0.23	11-15-79	≤0.10
SR3	4-3-79	≤0.10	5-14-79	16.55	6-25-79	0.20	11-15-79	≤0.10
SR3A	4-3-79	(a)	5-14-79	11.15	6-25-79	0.18	11-15-79	≤0.10
SR4	4-3-79	0.32	5-14-79	0.11	6-25-79	0.11	11-15-79	≤0.10
SR5	4-3-79	≤0.10	5-14-79	0.15	6-25-79	0.11	11-15-79	≤0.10
SR6	4-3-79	≤0.10	5-14-79	≤0.10	6-25-79	≤0.10	11-15-79	≤0.10
WR1	4-10-79	≤0.10	5-14-79	0.23	8-8-79	≤0.10	11-16-79	≤0.10
WR2	4-10-79	≤0.10	5-14-79	0.26	8-8-79	≤0.10	11-16-79	≤0.10
WR3	4-10-79	≤0.10	5-14-79	0.16	8-8-79	≤0.10	11-16-79	≤0.10
WR4	4-10-79	≤0.10	5-14-79	0.20	8-8-79	≤0.10	11-16-79	0.13
WR5	4-10-79	≤0.10	5-14-79	0.18	8-8-79	≤0.10	11-16-79	≤0.10
MB1	4-10-79	≤0.10	5-30-79	≤0.10	8-8-79	≤0.10	11-16-79	≤0.10
MB2	4-10-79	≤0.10	5-30-79	≤0.10	8-8-79	≤0.10	11-16-79	≤0.10
MB3	4-10-79	≤0.10	5-30-79	≤0.10	8-8-79	≤0.10	11-16-79	≤0.10
RR1	4-19-79	≤0.10	6-8-79	3.58	8-10-79	0.42	12-4-79	0.15
RR2	4-19-79	≤0.10	6-8-79	0.79	8-10-79	1.10	12-4-79	0.11
RR3	4-19-79	≤0.10	6-8-79	≤0.10	8-10-79	0.39	12-4-79	≤0.10
RR4	4-19-79	≤0.10	6-8-79	0.12	8-10-79	0.25	12-4-79	≤0.10
RR5	4-19-79	≤0.10	6-8-79	≤0.10	8-10-79	0.13	12-4-79	≤0.10

(continued)

TABLE 3.1. (continued)

	Circuit #1		Circuit #2		Circuit #3		Circuit #4	
Station	Date	Atrazine	Date	Atrazine	Date	Atrazine	Date	Atrazine
PR1	4-9-79	≤0.10	6-8-79	0.20	8-10-79	0.53	12-4-79	0.28
PR2	4-9-79	≤0.10	6-8-79	≤0.10	8-10-79	0.21	12-4-79	0.27
PR3	4-9-79	≤0.10	6-8-79	0.14	8-10-79	0.12	12-4-79	0.28
PR4	4-9-79	≤0.10	6-8-79	≤0.10	8-10-79	≤0.10	12-4-79	0.31
ES1	4-16-79	≤0.10	6-12-79	0.71	8-16-79	≤0.10	10-25-79	≤0.10
ES2	4-26-79	≤0.10	6-12-79	0.62	8-16-79	≤0.10	10-25-79	≤0.10
ES3	4-26-79	≤0.10	6-12-79	≤0.10	8-16-79	≤0.10	10-25-79	≤0.10
ES4	4-26-79	≤0.10	6-12-79	0.12	8-16-79	≤0.10	10-25-79	≤0.10
ES5	4-25-79	≤0.10	6-12-79	0.12	8-16-79	≤0.10	10-25-79	0.13
ES6	4-25-79	≤0.10	6-12-79	0.12	8-16-79	≤0.10	10-25-79	≤0.10
ES7	4-25-79	≤0.10	6-12-79	≤0.10	8-16-79	≤0.10	10-25-79	0.17
ES8	4-25-79	≤0.10	6-12-79	≤0.10	8-16-79	≤0.10	10-25-79	≤0.10

(a) station not initially occupied
(b) station occupied by bee swarm

TABLE 3.2. CONCENTRATIONS OF ATRAZINE IN SELECTED SEDIMENT SAMPLES FROM LOWER CHESAPEAKE BAY DURING 1979
(all concentrations in parts per billion; based on dry weight)

	Circuit #1		Circuit #2		Circuit #3		Circuit #4	
Station	Date	Atrazine	Date	Atrazine	Date	Atrazine	Date	Atrazine
SR1	4-3-79	33.83	5-14-79	≤5.0	6-25-79	25.13	11-15-79	21.33
SR2	4-3-79	N.Q.*	5-14-79	N.Q.*	6-25-79	35.08	11-15-79	13.01
SR3	4-3-79	≤5.0	5-14-79	N.Q.*	6-25-79	≤5.0	11-15-79	13.71
SR4	4-3-79	≤5.0	5-14-79	Lost	6-25-79	≤5.0	11-15-79	≤5.0**
SR5	4-3-79	≤5.0	5-14-79	≤5.0	6-25-79	≤5.0	11-15-79	5.38
SR6	4-3-79	≤5.0	5-14-79	≤5.0	6-25-79	≤5.0	11-15-79	≤5.0**
WR1	4-10-79	≤5.0	5-14-79	≤5.0	8-8-79	≤5.0	11-16-79	≤5.0
WR2	4-10-79	≤5.0	5-14-79	≤5.0	8-8-79	≤5.0	11-16-79	≤5.0
WR3	4-10-79	≤5.0	5-14-79	≤5.0	8-8-79	≤5.0	11-16-79	≤5.0
MB1	4-10-79	≤5.0	5-14-79	≤5.0	8-8-79	≤5.0	11-16-79	≤5.0
MB2	4-10-79	≤5.0	5-14-79	≤5.0	8-8-79	≤5.0	11-16-79	≤5.0
MB3	4-10-79	≤5.0	5-14-79	≤5.0	8-8-79	≤5.0	11-16-79	≤5.0

* not quantitatable due unresoluable interferences
** trace

well below 1 ppb. For the rest of the year atrazine concentrations were near or below the detection limit.

All three Mobjack Bay stations currently support extensive beds of submerged vegetation. These beds have apparently been relatively stable through the recent past. Atrazine concentrations in water samples were always below the detection limit.

None of the Rappahannock River stations currently support submerged vegetation. There were extensive beds of vegetation reported in the lower river in the early 1970's. The three down-river stations in this system are all apparently at sites which once supported submerged vegetation. Water sample analysis found atrazine concentrations below the detection limit prior to field applications in spring. Following application, atrazine was found in excess of 1 ppb at Port Royal, with concentrations generally decreasing to the detection limit at the river mouth. Atrazine was detected throughout the river system in late summer, with concentrations well below 1 ppb at the three down-river stations. Concentrations at those stations were below the detection limit during the final survey round.

In the Potomac River system none of the stations support *Zostera*. The upriver station at the Potomac River Bridge supports an extensive bed of *Potomageton perfoliatus* and *Vallisneria americana*. Water sample analyses found atrazine concentrations below the detection limit prior to spring field applications. Atrazine concentrations were highest at the Potomac River Bridge station in survey rounds two and three. Concentrations were relatively uniform throughout the river in survey round four. All concentrations, however, were well below 1 ppb.

Most of the Eastern Shore stations are in areas which currently support, or recently supported, submerged vegetation. Despite the intense agricultural land use on the Eastern Shore, water samples generally contained very little atrazine. No concentrations above 1 ppb were detected.

The principal objective of the 1979 survey program was to identify concentrations of atrazine potentially impacting *Zostera marina* in the lower Chesapeake Bay. The sampling program was designed to include those periods we believed, *a priori*, would include the maximum concentrations in Bay waters, i.e. immediately after field applications and shortly after harvesting. These two times should correspond with maximum runoff of sediments and chemicals from the fields.

One type of analysis of the data collected in the 1979 survey has been suggested by Dr. D. Leav (personal communication). Dr. Leav correctly observes that there is no assurance that the atrazine concentrations detected in this survey program are "worst case" concentrations given the frequency of the sampling. A conservative analysis of the data (i.e. one which ignores much of the information content of the sampling design) would compare the presence or absence of atrazine at each station with the loss or retention of submerged aquatic vegetation at that station. This approach reduces the information to a binominal data set with atrazine as a treatment. Analysis of those stations for which a good record of submerged aquatic vegetation occurrence is available indicates that vegetation has persisted in the presence of atrazine at four stations and disappeared at six stations. At stations where atrazine was not detected during the survey, vegetation has persisted at eleven stations and disappeared at one station. This analysis is suggestive of a correlation between the presence of atrazine and the disappearance of submerged aquatic vegetation. Unfortunately, there are insufficient numbers of samples in each treatment response category to allow a test of the significance of this correlation (x^2 test requires a minimum of 5 samples per category).

The analysis of the survey data we have employed for this report is "observational" and based on the assumption that the sampling was in fact representative of conditions in the lower Chesapeake Bay. With this assumption the survey results indicate several things.

First, atrazine concentrations in the lower Chesapeake Bay waters are generally below 1 ppb. Second, concentrations of atrazine above 1 ppb in water seem related to runoff events following spring application of herbicides. Third, in every case in the survey program, concentrations above 1 ppb were only found in upriver stations well removed from present or former Zostera beds. Fourth, concentrations of atrazine in waters over existing or former Zostera beds was generally 0.2 ppb or less.

REFERENCES

Orth, R. J., K. A. Moore, and H. H. Gordon. 1979. Distribution and abundance of submerged aquatic vegetation in the lower Chesapeake Bay, Virginia. U.S. EPA Final Report. 600/8-79-029/SAV1. 199 pp.

TABLE A3.1. 1979 VIRGINIA SURVEY SITES

Chesapeake Bay

CB-1, Dameron Marsh
coordinates: latitude 37°47'14", longitude 76°18'16"
location: southside of Ingram Bay, opposite Fleeton, north west corner of Dameron Marsh, 200 meters offshore (USGS Reedville Quadrangle)
access: Rt. 606, private lane and wading
depth: 1 meter
sediment: sand
SAV: none

CB-2, Fleets Bay
coordinates: latitude 37°39'36", longitude 76°20'10"
location: Fleets Bay, end of Poplar Neck, midway between Dymer Creek and Tabbs Creek, 5 meters offshore just south of unnamed impoundment (USGS Fleets Bay Quadrangle)
access: Rt. 646, Rt. 647, private lane and wading
depth: 1 meter
sediment: sand
SAV: none

CB-3, Cricket Hill/Gwynn's Island
coordinates: latitude 37°29'12", longitude 76°18'5"
location: Milford Haven, northwest shore at mouth of Lanes Creek, southside of land at terminus of Rt. 669 in Cricket Hill, 10 meters offshore (USGS Mathews Quadrangle)
access: Rt. 223, Rt. 669 and wading
depth: 1 meter
sediment: sand and silt
SAV: none

CB-4, Plum Tree Island
coordinates: latitude 37°10'35", longitude 76°25'24"
location: south of Poquoson River, 50 meters offshore of north east terminus of Plum Tree Island bombing range (identified as Marsh Point) (USGS Poquoson East Quadrangle)
access: boat
depth: 1.3 meter
sediment: sand
SAV: none

James River

JR-1, Hopewell
coordinates: latitude 37°18'55", longitude 77°13'7"
location: southshore, 200 meters upstream of Benjamin Harris Bridge, 15 meters offshore (USGS Westover Quadrangle)
access: Rt. 156 and wading

(continued)

TABLE A3.1. (continued)

depth: 1 meter
sediment: sand and silt
SAV: none

JR-2, Chickahominy
coordinates: latitude 37°14'22", longitude 76°51'55"
location: north shore, 500 meters downstream of Chickahominy River mouth, 50 meters offshore (USGS Surry Quadrangle)
access: boat
depth: 1 meter
sediment: sand, clay and silt
SAV: none

JR-3, James River Bridge
coordinates: latitude 36°57'40", longitude 76°30'51"
location: south shore, 100 meters downstream of James River Bridge, 20 meters offshore (USGS Benn's Church Quadrangle)
access: Rt. 17 and wading
depth: 1 meter
sediment: sand and silt
SAV: none

York River

YR-1A, Sweet Hall Marsh
coordinates: latitude 37°34'10", longitude 76°54'28"
location: Pamunky River, northshore, 50 meters upstream of impoundment outfall at Sweet Hall Landing, 10 meters offshore (USGS New Kent Quadrangle)
access: Rt. 634 and wading
depth: 1 meter
sediment: sand,gravel and silt
SAV: none

YR-1B, Water Fence Landing
coordinates: latitude 37°35'30", longitude 76°47'57"
location: Mattaponi River, northshore, public boat ramp at Water Fence Landing, 5 meters offshore (USGS West Point Quadrangle)
access: Rt. 611 and wading
depth: 1 meter
sediment: silt
SAV: none

YR-1C, Gressitt
coordinates: latitude 37°28'0", longitude 76°43'35"
location: north shore of York River, 3100 meters upstream of Propotank River, 100 meters offshore (USGS Gressitt Quadrangle)
access: Rt. 667 and wading
depth: 1 meter
sediment: sand and silt

(continued)

TABLE 43.1. (continued)
SAV: none

YR-1, Carter Creek
coordinates: latitude 37°19'22", longitude 76°34'24"
location: north shore of Carter Creek, 1000 meters upstream of mouth (Blundering Point), 20 meters offshore (USGS Clay Bank Quadrangle)
access: boat
depth: 1 meter
sediment: clay and silt
SAV: none

YR-2, Mumfort Island
coordinates: latitude 37°16'6", longitude 76°31'0"
location: north shore of York River, south west of southern Mumfort Island, 1800 meters north of Gloucester Point, 50 meters offshore of island (USGS Clay Bank Quadrangle)
access: boat
depth: 1 meter
sediment: sand, clay and silt
SAV: none

YR-3, Allen's Island
coordinates: latitude 37°15'25", longitude 76°25'20"
location: north shore of York River, 50 meters off south shore of island (USGS Achilles Quadrangle)
access: boat
depth: 1 meter
sediment: sand and silt
SAV: *Zostera marina*

YR-4, Guinea Marsh
coordinates: latitude 37°16'24", longitude 76°20'44"
location: north side of York River mouth, 800 meters east-south east of last Guinea Marsh island (USGS New Point Comfort Quadrangle)
access: boat
depth: 1.3 meter
sediment: sand and silt
SAV: *Zostera marina*

YR-5, Browns' Bay
coordinates: latitude 37°18'2", longitude 76°23'39"
location: east of Blevins Creek mouth, 10 meters offshore (USGS Achilles Quadrangle)
access: boat
depth: 1.3 meter
sediment: sand and silt
SAV: *Zostera marina*

Severn River

(continued)

TABLE A3.1. (continued)

SR-1, Warner Hall north drainage
coordinates: latitude 37°20'39", longitude 76°29'6"
location: northwest branch of Severn River, head of northern most tributary, paralleling Rt. 629, 3100 meters upstream of Bray's landing (USGS Achilles Quadrangle)
access: boat
depth: 0.5 meter
sediment: silt and clay
SAV: none

SR-1A, Warner Hall north drainage
coordinates: latitude 37°20'28", longitude 76°29'0"
location: northwest branch of Severn River, mid-axis of northernmost tributary paralleling Rt. 629, 250 meters downstream from SR-1 (USGS Achilles Quadrangle)
access: boat
depth: 0.5 meters
sediment: silt and clay
SAV: none

SR-1B, Warner Hall, Severn River headwater
coordinates: latitude 37°20'20", longitude 76°28'56"
location: northwest branch of Severn River, mouth of northernmost tributary, 500 meters downstream of SR-1 (USGS Achilles Quadrangle)
access: boat
depth: 0.5 meters
sediment: silt and clay
SAV: none

SR-2, Warner Hall cemetery
coordinates: latitude 37°20'14", longitude 76°28'37"
location: northwest branch of Severn River, east shore, small inlet 2000 meters upstream of Brays Landing, due south of Warner Hall cemetery, 5 meters offshore (USGS Achilles Quadrangle)
access: boat or Rt. 629 and wading
depth: 0.5 meters
sediment: silt and clay
SAV: none

SR-2A, Warner Hall cemetery (2)
coordinates: latitude 37°20'5", longitude 76°28'40"
location: northwest branch of Severn River, 2000 meters upstream of Brays Landing, west of SR2, 15 meters offshore (USGS Achilles Quadrangle)
access: boat
depth: 0.5 meters
sediment: silt and clay
SAV: none

SR-3, Eagle Point west drainage

(continued)

TABLE A3.1. (continued)

coordinates: latitude 37°19'54", longitude 76°28'15"
location: north west branch of Severn River, east shore, small inlet 1100 meters upstream of Brays Landing, 10 meters offshore (USGS Achilles Quadrangle)
access: boat
depth: 0.5 meters
sediment: silt and clay
SAV: none

SR-3A, Severn River northwest channel
coordinates: latitude 37°19'37", longitude 76°28'24"
location: northwest branch of Severn River, main axis, 800 meters upstream of Brays Landing, 10 meters offshore (USGS Achilles Quadrangle)
access: boat
depth: 0.7 meters
sediment: silt and clay
SAV: none

SR-4, Cod Point
coordinates: latitude 37°19'23", longitude 76°27'18"
location: northwest branch of Severn River, north shore, westend of Bryant Bay, end of Cod Point, 15 meters offshore (USGS Achilles Quadrangle)
access: boat
depth: 1 meter
sediment: silt, sand and clay
SAV: none

SR-5, School Neck Point
coordinates: latitude 37°19'21", longitude 76°26'29"
location: northwest branch of Severn River, north shore, eastend of Bryant Bay, 25 meters offshore of School Neck Point (USGS Achilles Quadrangle)
access: boat
depth: 1 meter
sediment: sand and silt
SAV: none

SR-6, Turtle Neck Point
coordinates: latitude 37°19'18", longitude 76°25'15"
location: northshore of Severn River, mouth of river, 200 meters offshore southwest of Turtle Neck Point (USGS Achilles Quadrangle)
access: boat
depth: 1 meter
sediment: sand and clay
SAV: *Ruppia maritima*

Ware River

WR-1, Goshen

(continued)

TABLE A3.1. (continued)

coordinates: latitude 37°23'54", longitude 76°29'15"
location: south shore of Ware River, 600 meters downstream of public landing at end of Deacon's Neck, 25 meters offshore (USGS Ware Neck Quadrangle)
access: boat
depth: 1 meter
sediment: silt and clay
SAV: none

WR-2, Bailey's Wharf
coordinates: latitude 37°23'15", longitude 76°27'48"
location: south shore of Ware River, 25 meters offshore of northside of Bailey's Wharf (USGS Ware Neck Quadrangle)
access: boat
depth: 1 meter
sediment: silt and clay
SAV: none

WR-3, Wilson Creek
coordinates: latitude 37°21'57", longitude 76°28'8"
location: south shore of Ware River, mouth of Wilson Creek, 50 meters offshore of west side of Roanes Wharf (USGS Achilles Quadrangle)
access: boat
depth: 1 meter
sediment: sand and silt
SAV: *Ruppia maritima*

WR-4, Windmill Point
coordinates: latitude 37°21'57", longitude 76°26'51"
location: south shore of Ware River, north of Oldhouse Creek mouth, 50 meters offshore of west side of Windmill Point (USGS Achilles Quadrangle)
access: boat
depth: 1 meter
sediment: sand
SAV: *Zostera marina* and *Ruppia maritima*

WR-5, Four Point Marsh
coordinates: latitude 37°20'30", longitude 76°24'34"
location: south shore of Ware River, mouth of river, between Ware River Point and Tow Stake Point on Four Point Marsh, 300 meters south of Ware River Point (USGS Achilles Quadrangle)
access: boat
depth: 1 meter
sediment: sand
SAV: *Zostera marina* and *Ruppia maritima*

Mobjack Bay

MB-1, Whites Neck

(continued)

TABLE A3.1. (continued)

coordinates: latitude 37°22'5", longitude 76°21'15"
location: northeast shore of Mobjack Bay, between North River and East River, 100 meters offshore between Minter Point and Pond Point at southern end of Whites Neck (USGS New Point Comfort Quadrangle)
access: boat
depth: 1 meter
sediment: sand
SAV: *Zostera marina* and *Ruppia maritima*

MB-2, Bay Shore Point
coordinates: latitude 37°21'42", longitude 76°20'20"
location: northeast shore of Mobjack Bay, south of East River mouth, 200 meters offshore, 500 meters south of Bay Shore Point (USGS New Point Comfort Quadrangle)
access: boat
depth: 1 meter
sediment: sand
SAV: *Zostera marina* and *Ruppia maritima*

MB-3, Pepper Creek
coordinates: latitude 37°20'26", longitude 76°19'53"
location: northeast shore of Mobjack Bay south shore of Pepper Creek at mouth, 150 meters offshore (USGS New Point Comfort Quadrangle)
access: boat
depth: 1 meter
sediment: sand
SAV: *Zostera marina* and *Ruppia maritima*

Rappahannock River

RR-1, Port Royal
coordinates: latitude 38°10'34", longitude 77°11'12"
location: north shore of river, east side of Rt. 301 bridge, 5 meters offshore (USGS Port Royal Quadrangle)
access: Rt. 301 and wading
depth: 1 meter
sediment: silt and clay
SAV: none

RR-2, Tappahannock
coordinates: latitude 37°56'22", longitude 76°50'33"
location: north shore of river, east side of Rt. 360 bridge, 5 meters offshore (USGS Tappahannock Quadrangle)
access: Rt. 360 and wading
depth: 1 meter
sediment: sand
SAV: none

RR-3, Butylo
coordinates: latitude 37°46'6", longitude 76°40'57"

(continued)

TABLE A3.1. (continued)

location: south shore of river, McKans Bay, north side of causeway to marmade island, 200 meters offshore (USGS Morattico Quandrangle)
access: Rt. 600 and wading
depth: 1 meter
sediment: silt
SAV: none

RR-4, Rosegill Farm
coordinates: latitude 37°38'2", longitude 76°33'33"
location: south shore of river, 1000 meters downstream from Bailey Point at mouth of Urbanna Creek, 25 meters offshore from dam forming Rosegill Lake (USGS Urbanna Quadrangle)
access: Rt. 227, private lane and wading
depth: 1 meter
sediment: sand and clay
SAV: none

RR-5, Stingray Point
coordinates: latitude 37°33'21", longitude 76°17'59"
location: mouth of river, southern shore, 500 meters south of Stingray Point, 5 meters offshore (USGS Deltaville Quadrangle)
access: Rt. 33 and wading
depth: 1 meter
sediment: sand
SAV: none

Potomac River

PR-1, Potomac River Bridge
coordinates: latitude 38°21'38", longitude 77°0'52"
location: south shore of river, 300 meters upstream from Rt. 301 bridge, 25 meters offshore (USGS Dahlgren Quadrangle)
access: Rt. 301 and wading
depth: 1 meter
sediment: sand and clay
SAV: *Potamogeton perfoliatus* and *Vallisneria americana*

PR-2, Ragged Point
coordinates: latitude 38°8'32", longitude 76°36'50"
location: south shore of river, 800 meters south of Ragged Point, just north of Long Pond, former Pond-a-River Campground, 10 meters offshore (USGS Piney Point Quadrangle)
access: Rt. 728 and wading
depth: 1 meter
sediment: sand
SAV: none

PR-3, Coan River
coordinates: latitude 37°50'10", longitude 76°27'0"

(continued)

TABLE A3.1. (continued)

location: south shore of river, south of Coan River mouth, 1200 meters east of Walnut Point, 250 meters west of Balls Creek mouth, 50 meters offshore (USGS Heathsville Quadrangle)
access: Rt. 630 and wading
depth: 1 meter
sediment: sand and clay
SAV: none

PR-4, Smith Point, Ginny Beach
coordinates: latitude 37°54'5", longitude 76°15'13"
location: south shore of river, 1850 meters upstream of Little Wicomico River mouth, 5 meters offshore (USGS Burgess Quadrangle)
access: Rt. 649 and wading
depth: 1 meter
sediment: sand
SAV: none

Eastern Shore

ES-1, Pocomoke River
coordinates: latitude 37°58'30", longitude 75°37'52"
location: south shore of river, between Pitts Creek and Bullbegger Creek, north side of Pitts Neck, public dock at end of Rt. 709 (USGS Saxis Quadrangle)
access: Rt. 709
depth: 1 meter
sediment: silt and clay
SAV: none

ES-2, Saxis
coordinates: latitude 37°56'10", longitude 75°43'5"
location: south shore of Pocomoke Sound, north of Saxis, south of North End Point, 20 meters offshore (USGS Saxis Quadrangle)
access: Rt. 695 and wading
depth: 1 meter
sediment: sand
SAV: none

ES-3 Chesconessex Creek
coordinates: latitude 37°45'1", longitude 75°47'36"
location: south of Chesconessex Creek, just north of unnamed inlet midway between Chesconessex Creek and Back Creek, 50 meters offshore (USGS Chesconessex Quadrangle)
access: Rt. 782 and wading
depth: 1 meter
sediment: sand
SAV: none

ES-4, Davis Wharf

(continued)

TABLE A3.1. (continued)

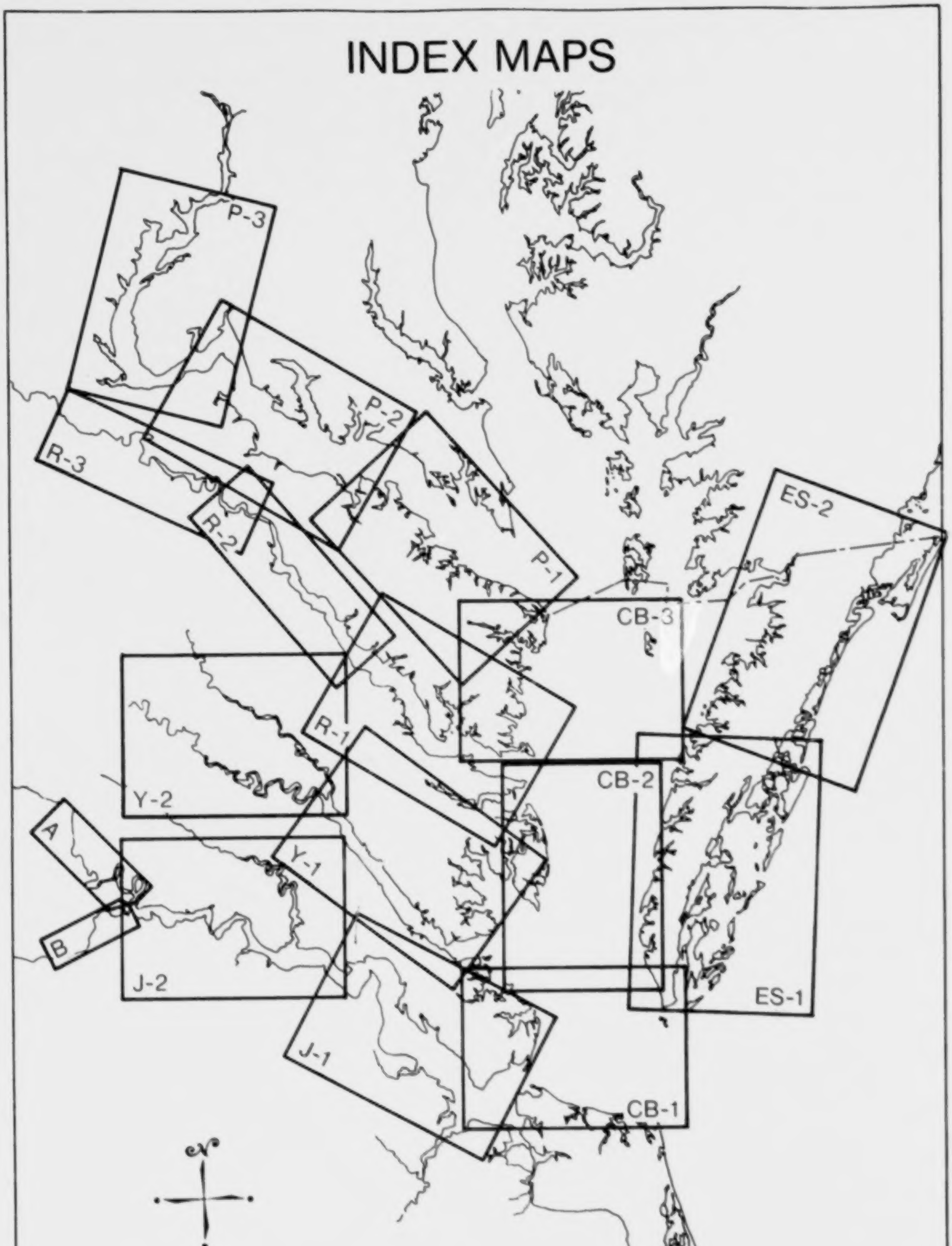
coordinates: latitude 37°33'3", longitude 75°52'44"
location: north shore of Occohannock Creek, due south of Davis Wharf, 25 meters offshore (USGS Jamesville Quadrangle)
access: Rt. 615 and wading
depth: 1 meter
sediment: sand and silt
SAV: none

ES-5, Occohannock Creek
coordinates: latitude 37°33'28", longitude 75°56'3"
location: north shore of Occohannock Creek near mouth, between Powells Bluff and Johns Point, 50 meters offshore of unnamed impoundment (USGS Jamesville Quadrangle)
access: Rt. 612, private lane and wading
depth: 1 meter
sediment: sand
SAV: none

ES-6, Vaucluse Shores
coordinates: latitude 37°24'18", longitude 75°59'6"
location: north of Hungars Creek mouth, 500 meters offshore from Great Neck (USGS Franktown Quadrangle)
access: boat
depth: 1.3 meters
sediment: sand and clay
SAV: *Zostera marina*

ES-7, Hungars Creek
coordinates: latitude 37°25'5", longitude 75°57'41"
location: mid-axis Hungars Creek, between Sparrow Point on north shore and Masden Gulf on south shore (USGS Franktown Quadrangle)
access: boat
depth: 1.3 meters
sediment: sand and clay
SAV: *Ruppia maritima*

ES-8, Picketts Harbor
coordinates: latitude 37°11'19" longitude 75°59'59"
location: north of Butlers Bluff on Chesapeake Bay shore, 10 meters offshore of old range tower at Picketts Harbor (USGS Townsend Quadrangle)
access: Rt. 646 and wading
depth: 1 meter
sediment: sand
SAV: none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N

Figure A3.1. Index maps for lower Chesapeake Bay survey stations.

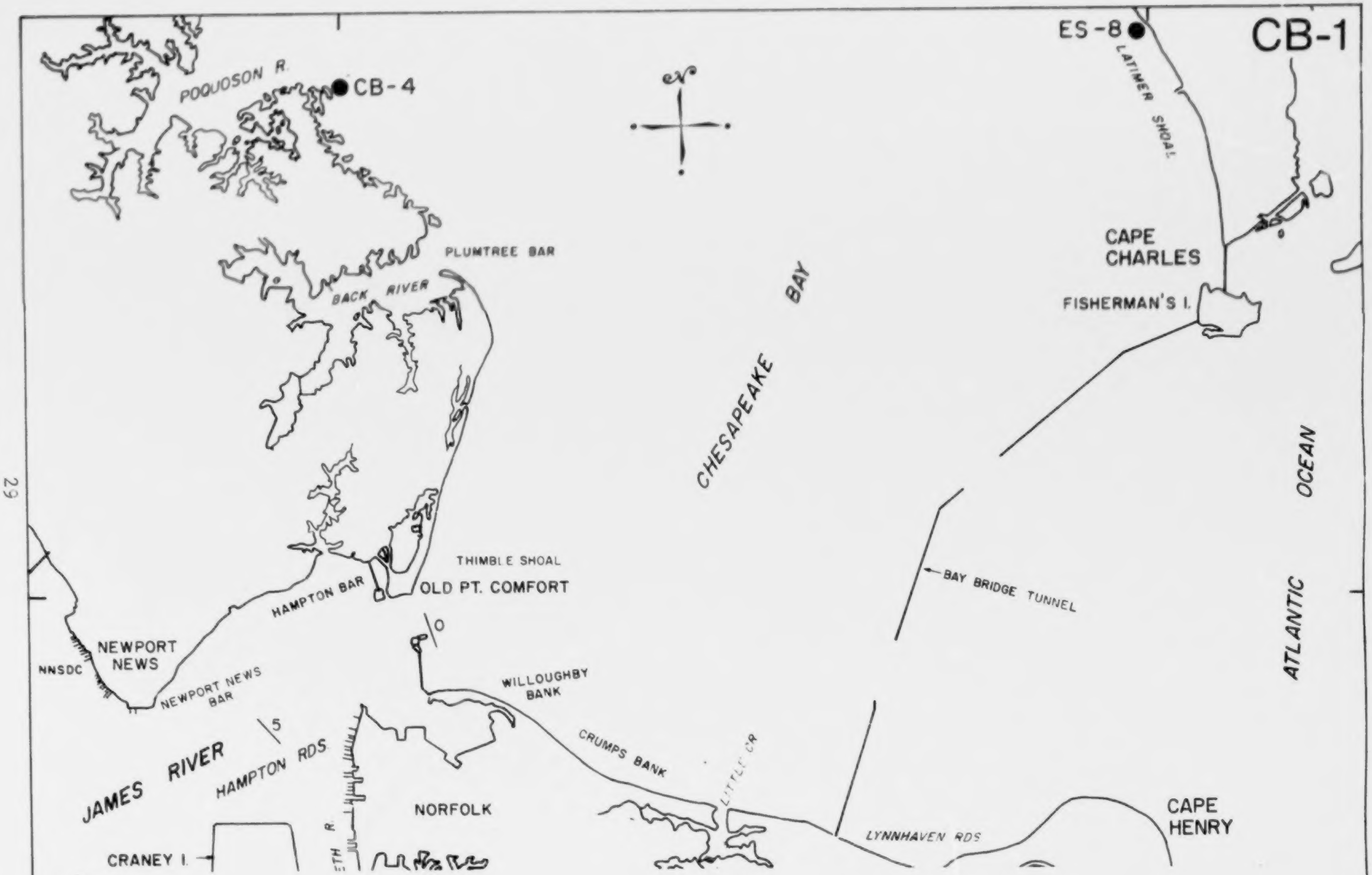
CB-1
ES-8
LATIMER SHOAL
CB-4
POQUOSON R.
PLUMTREE BAR
BACK RIVER
CAPE CHARLES
FISHERMAN'S I.
CHESAPEAKE BAY
ATLANTIC OCEAN
BAY BRIDGE TUNNEL
THIMBLE SHOAL
OLD PT. COMFORT
HAMPTON BAR
NEWPORT NEWS
NNSDC
NEWPORT NEWS BAR
WILLOUGHBY BANK
CRUMPS BANK
LITTLE CR
JAMES RIVER
HAMPTON RDS
NORFOLK
CRANEY I.
ETH R.
LYNNHAVEN RDS
CAPE HENRY
0
5

Figure A3.2. Locations of survey stations CB-4 (Plum Tree Island) and ES-8 (Picketts Harbor).

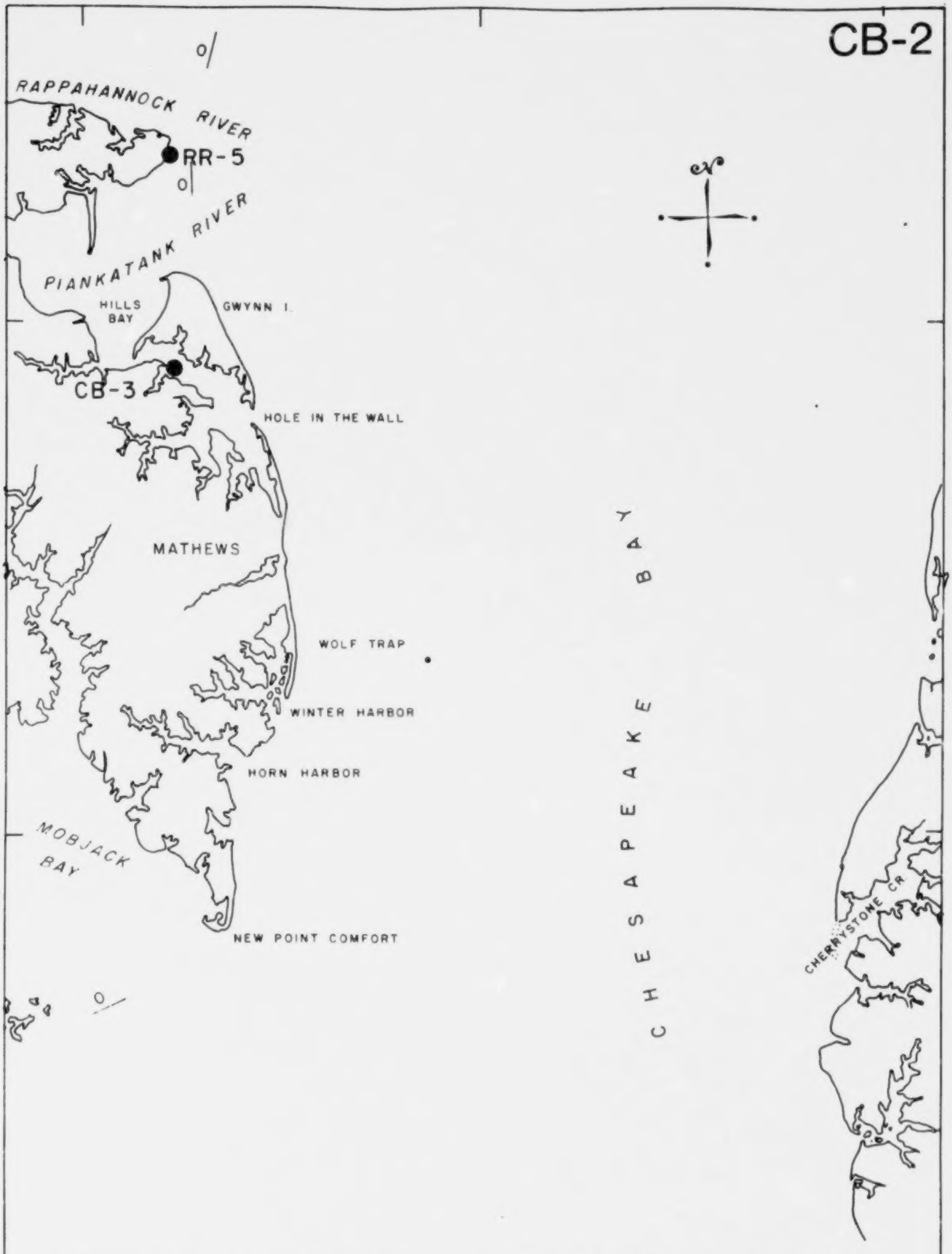
CB-2
RAPPAHANNOCK RIVER
RR-5
PIANKATANK RIVER
HILLS BAY
GWYNN I.
CB-3
HOLE IN THE WALL
MATHEWS
WOLF TRAP
WINTER HARBOR
HORN HARBOR
MOBJACK BAY
NEW POINT COMFORT
CHESAPEAKE BAY
CHERRYSTONE CR
N

Figure A3.3. Locations of survey stations RR-5 (Stingray Point) and CB-3 (Crickett Hill/Gwynn's Island).

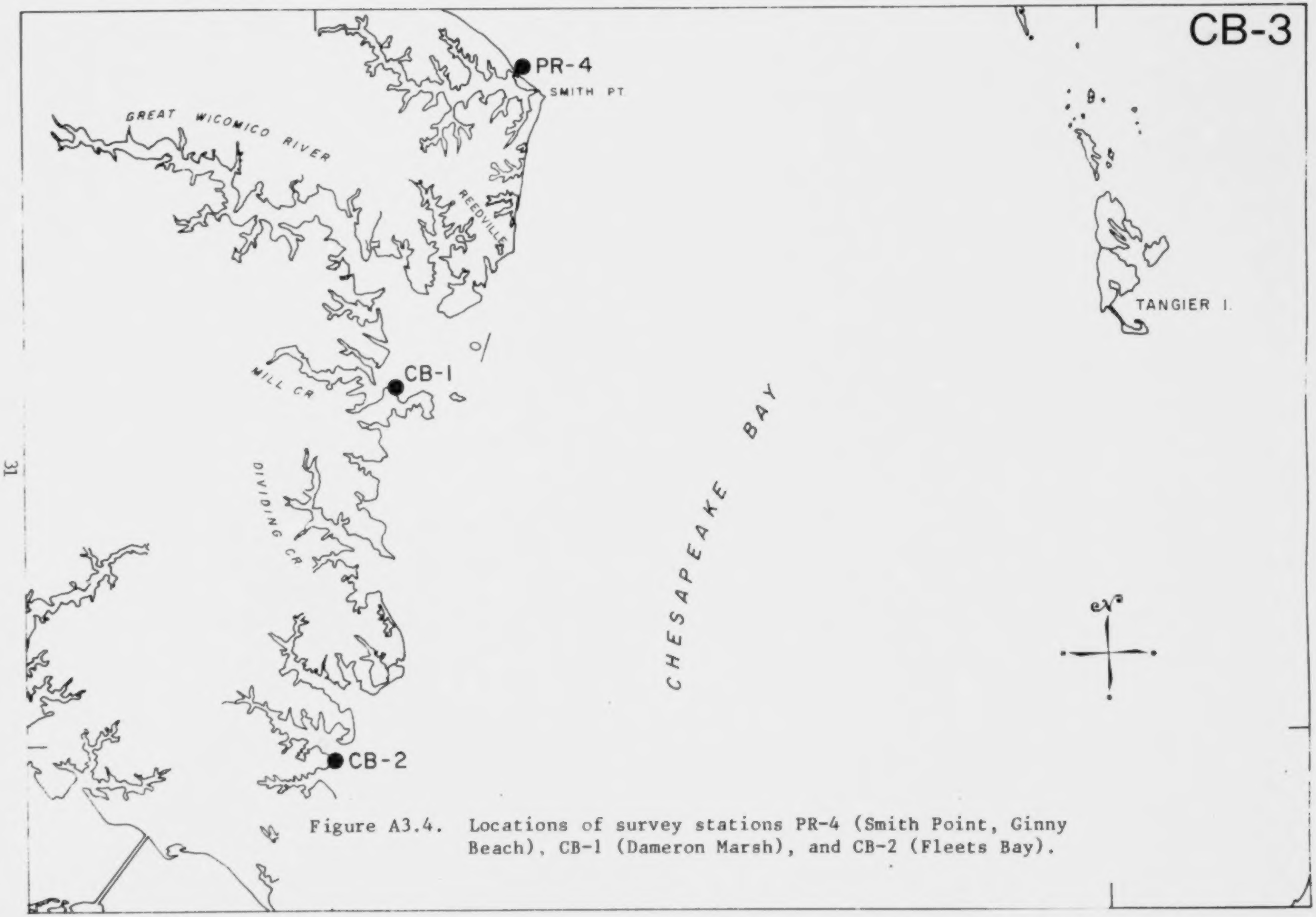
CB-3
PR-4
SMITH PT
GREAT WICOMICO RIVER
REEDVILLE
TANGIER I.
CB-1
MILL CR
DIVIDING CR
CHESAPEAKE BAY
CB-2

Figure A3.4. Locations of survey stations PR-4 (Smith Point, Ginny Beach), CB-1 (Dameron Marsh), and CB-2 (Fleets Bay).

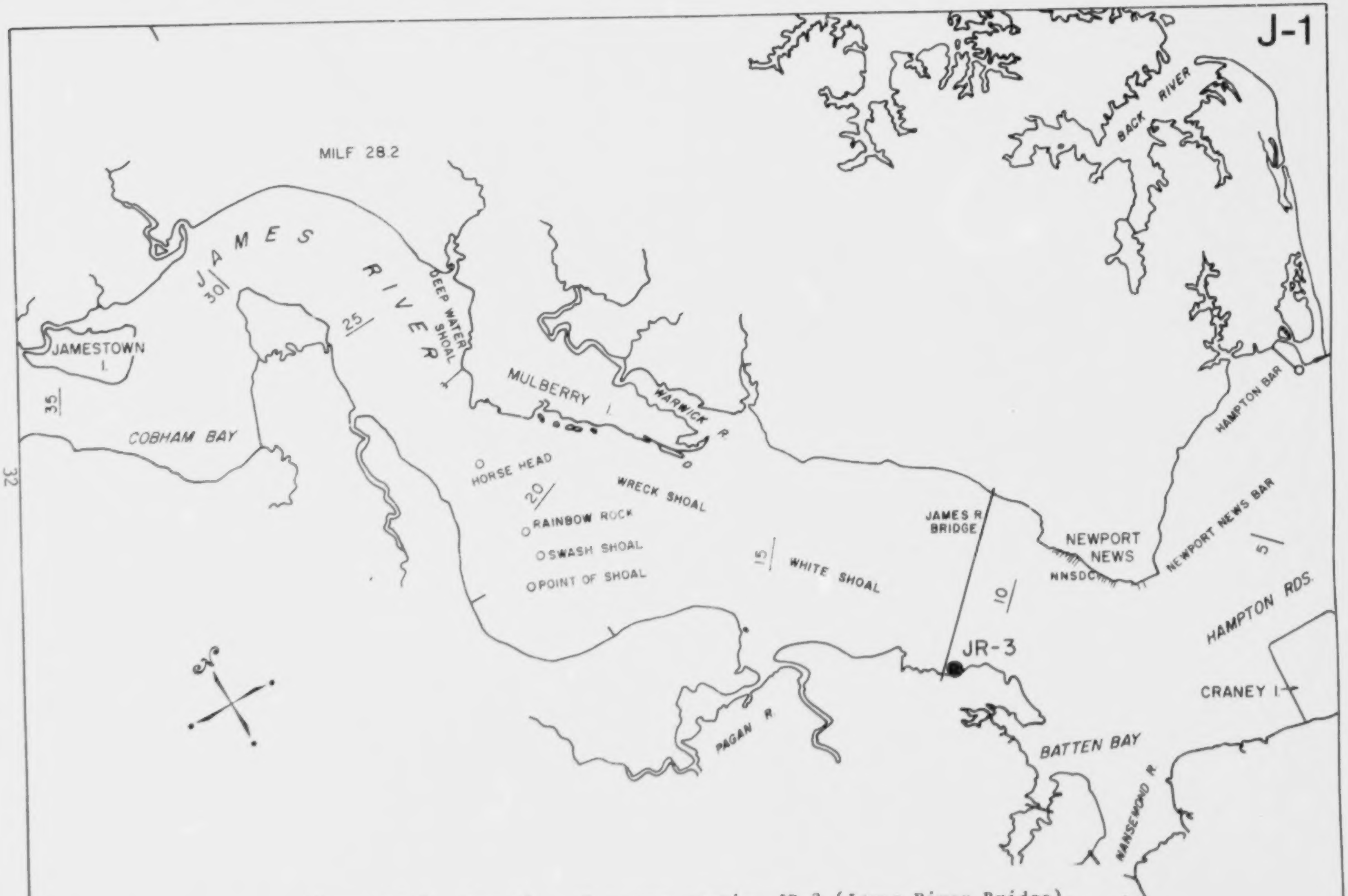
J-1
MILF 28.2
JAMES RIVER
JAMESTOWN I.
35
30
25
20
15
10
5
COBHAM BAY
DEEP WATER SHOAL
MULBERRY I.
WARWICK R.
HORSE HEAD
WRECK SHOAL
RAINBOW ROCK
SWASH SHOAL
POINT OF SHOAL
WHITE SHOAL
PAGAN R.
JAMES R. BRIDGE
JR-3
NEWPORT NEWS
NNSDC
NEWPORT NEWS BAR
HAMPTON BAR
BACK RIVER
HAMPTON RDS.
CRANEY I.
BATTEN BAY
NANSEMOND R.

Figure A3.5. Location of survey station JR-3 (James River Bridge).

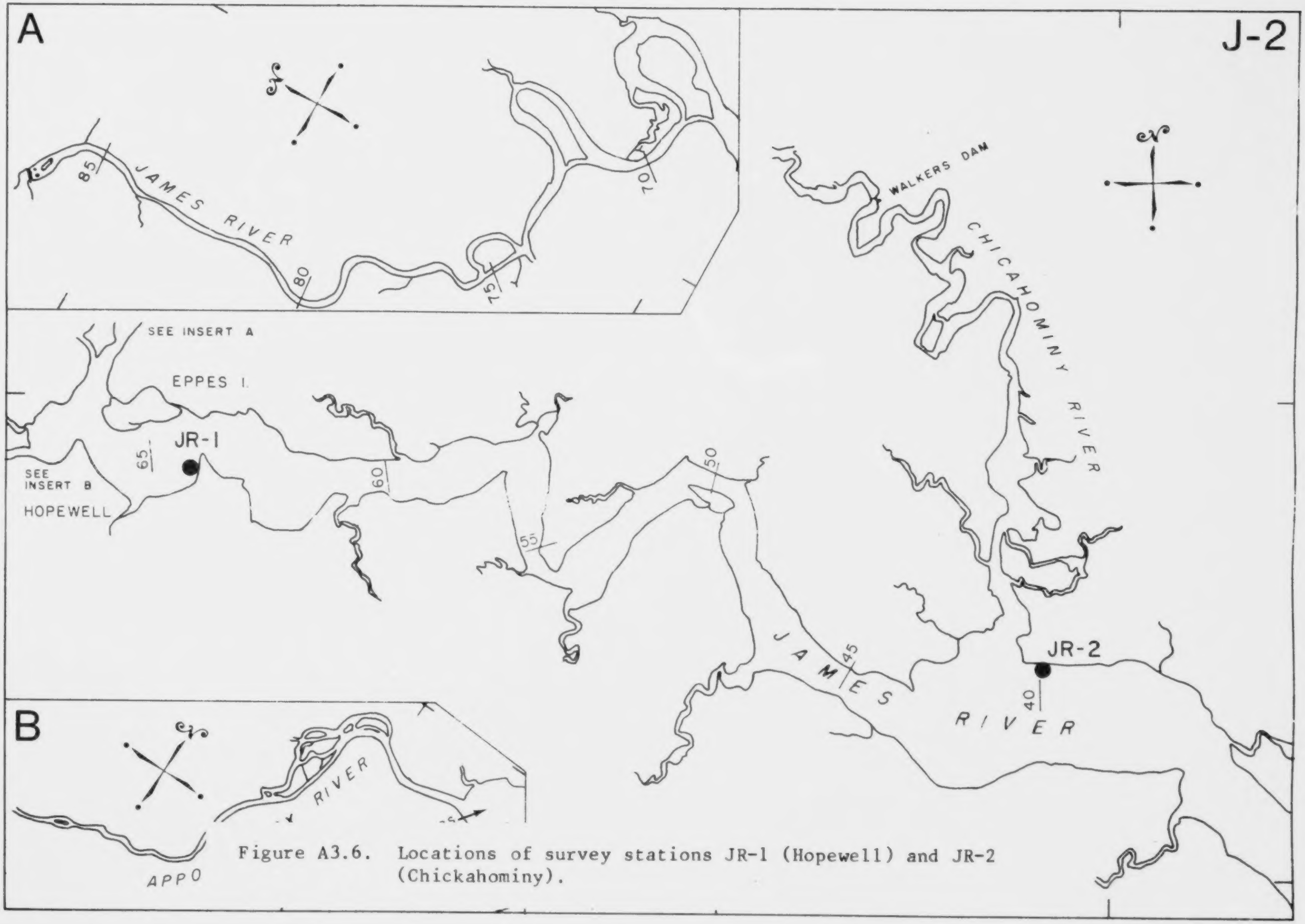
A
J-2
JAMES RIVER
85
80
75
70
SEE INSERT A
EPPES I.
JR-1
65
60
55
50
45
40
SEE INSERT B
HOPEWELL
WALKERS DAM
CHICAHOMINY RIVER
JAMES RIVER
JR-2
B
RIVER
APPO

Figure A3.6. Locations of survey stations JR-1 (Hopewell) and JR-2 (Chickahominy).

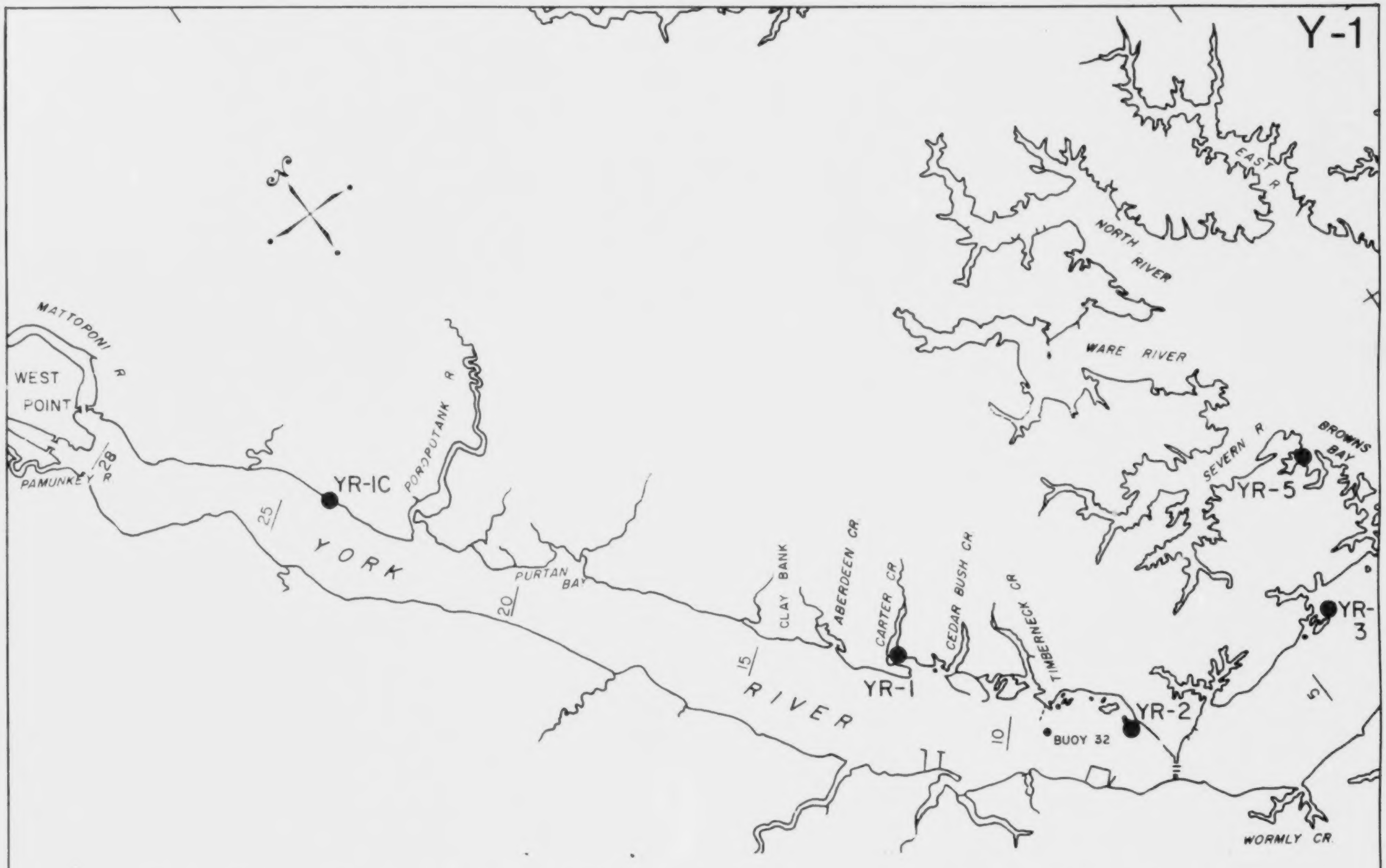
Y-1
EAST R
NORTH RIVER
WARE RIVER
SEVERN R
BROWNS BAY
YR-5
YR-3
MATTOPONI R
WEST POINT
PAMUNKEY R
28
PORoPUTANK R
YR-1C
25
YORK
PURTAN BAY
20
CLAY BANK
ABERDEEN CR
CARTER CR
CEDAR BUSH CR
TIMBERNECK CR
15
RIVER
YR-1
10
BUOY 32
YR-2
5
WORMLY CR

Figure A3.7. Locations of survey stations YR-1C (Gressitt), YR-1 (Carter Creek), YR-2 (Mumfort Island), YR-3 (Allens' Island), and YR-5 (Browns' Bay).

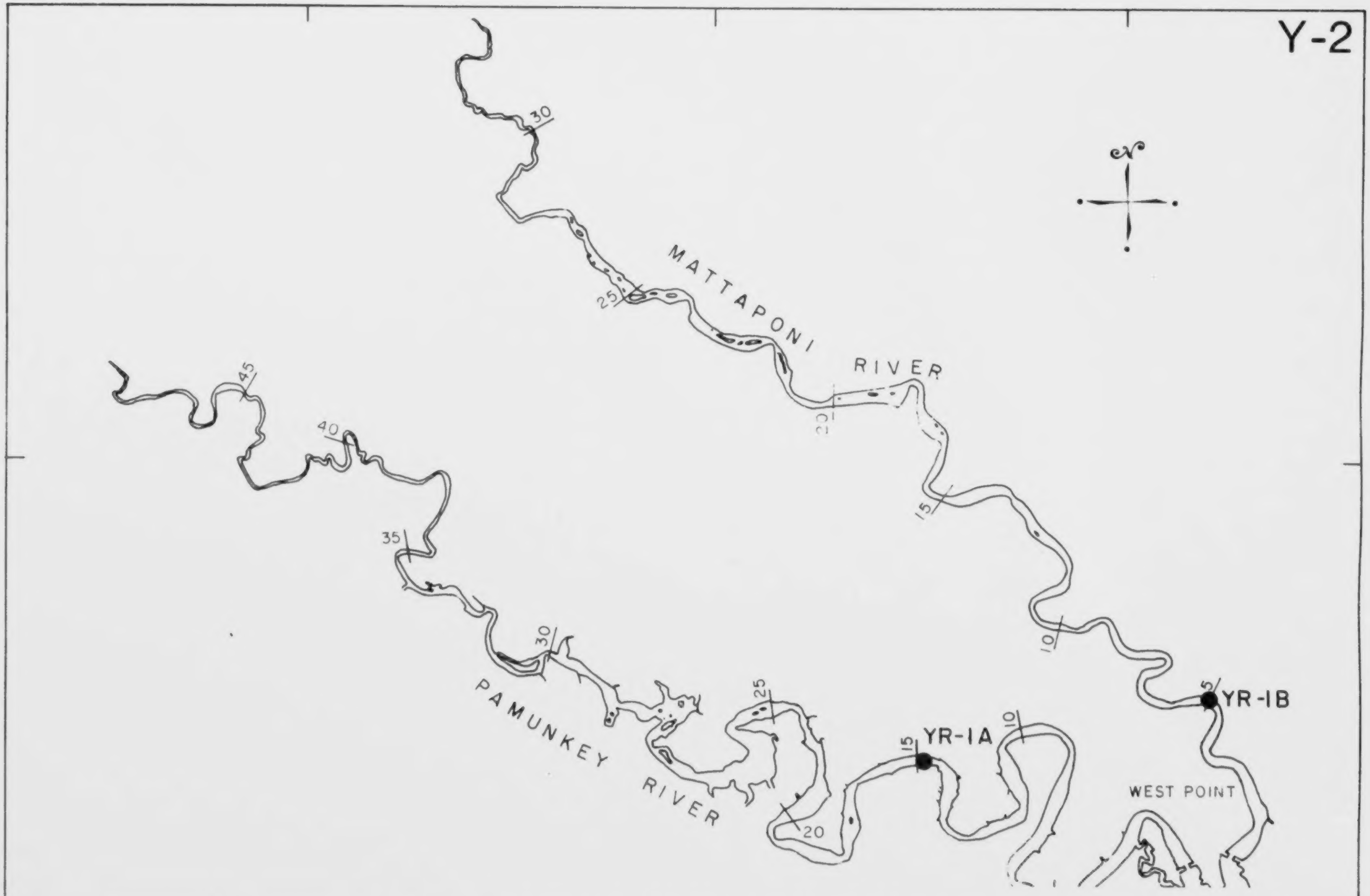
Y-2
MATTAPONI RIVER
PAMUNKEY RIVER
WEST POINT
YR-1B
YR-1A
5
10
15
20
25
30
35
40
45

Figure A3.8. Locations of survey stations YR-1A (Sweet Hall Marsh) and YR-1B (Water Fence Landing).

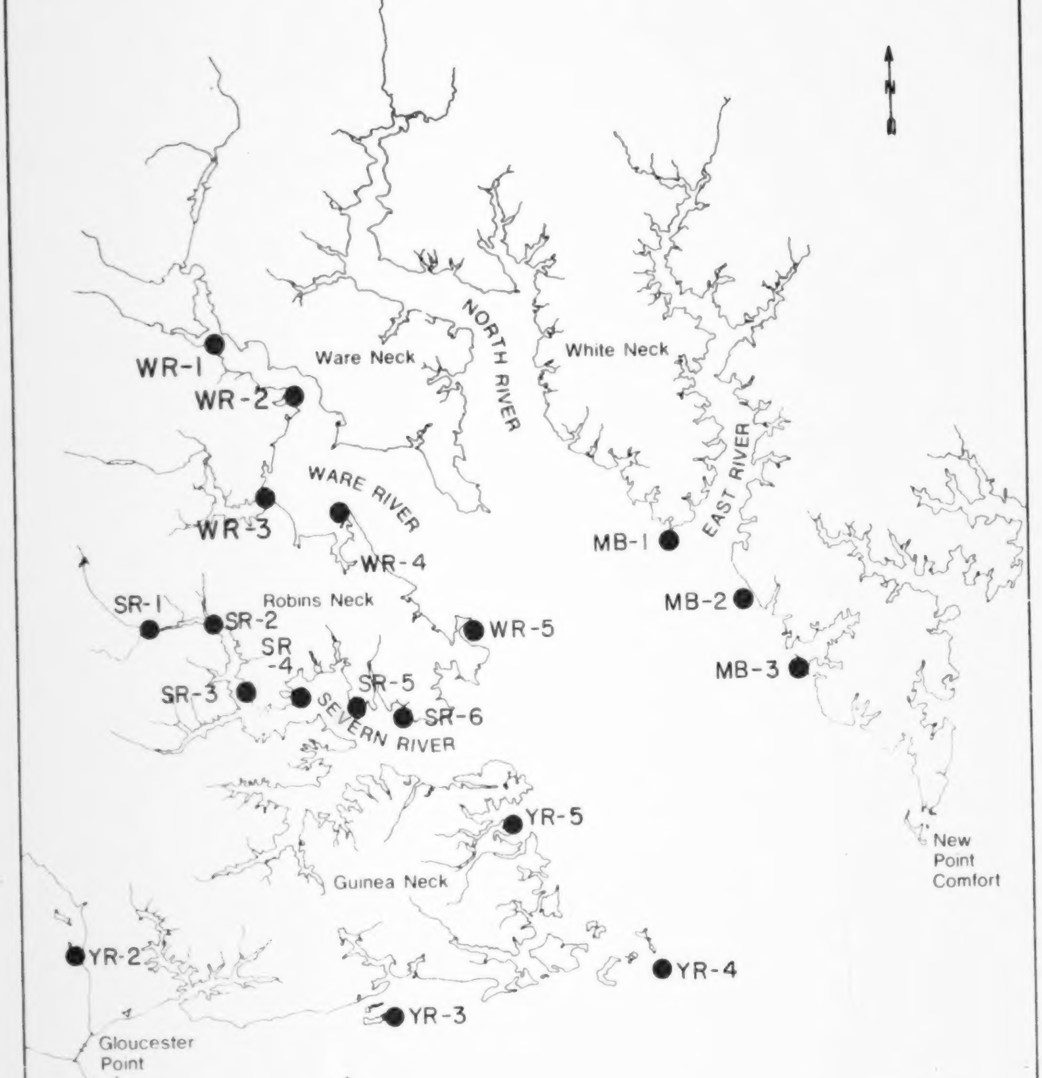
N
WR-1
WR-2
WR-3
WR-4
WR-5
Ware Neck
NORTH RIVER
White Neck
EAST RIVER
WARE RIVER
MB-1
MB-2
MB-3
Robins Neck
SR-1
SR-2
SR -4
SR-3
SR-5
SR-6
SEVERN RIVER
YR-5
Guinea Neck
New Point Comfort
YR-2
YR-4
YR-3
Gloucester Point

Figure A3.9. Locations of survey stations WR-1 (Goshen), WR-2 (Bailey's Wharf), WR-3 (Wilson Creek), WR-4 (Windmill Point), WR-5 (Four Point Marsh), MB-1 (Whites Neck Point), MB-2 (Bay Shore Point), MB-3 (Pepper Creek), SR-1 (Warner Hall Plantation north drainage), SR-2 (Warner Hall Cemetery), SR-3 (Eagle Point Plantation west drainage), SR-4 (Cod Point), SR-5 (School Neck Point), SR-6 (Turtle Neck Point), YR-2 (Mumfort Islands), YR-3 (Allen's Island), YR-4 (Guinea Marshes), and YR-5 (Brown's Bay).

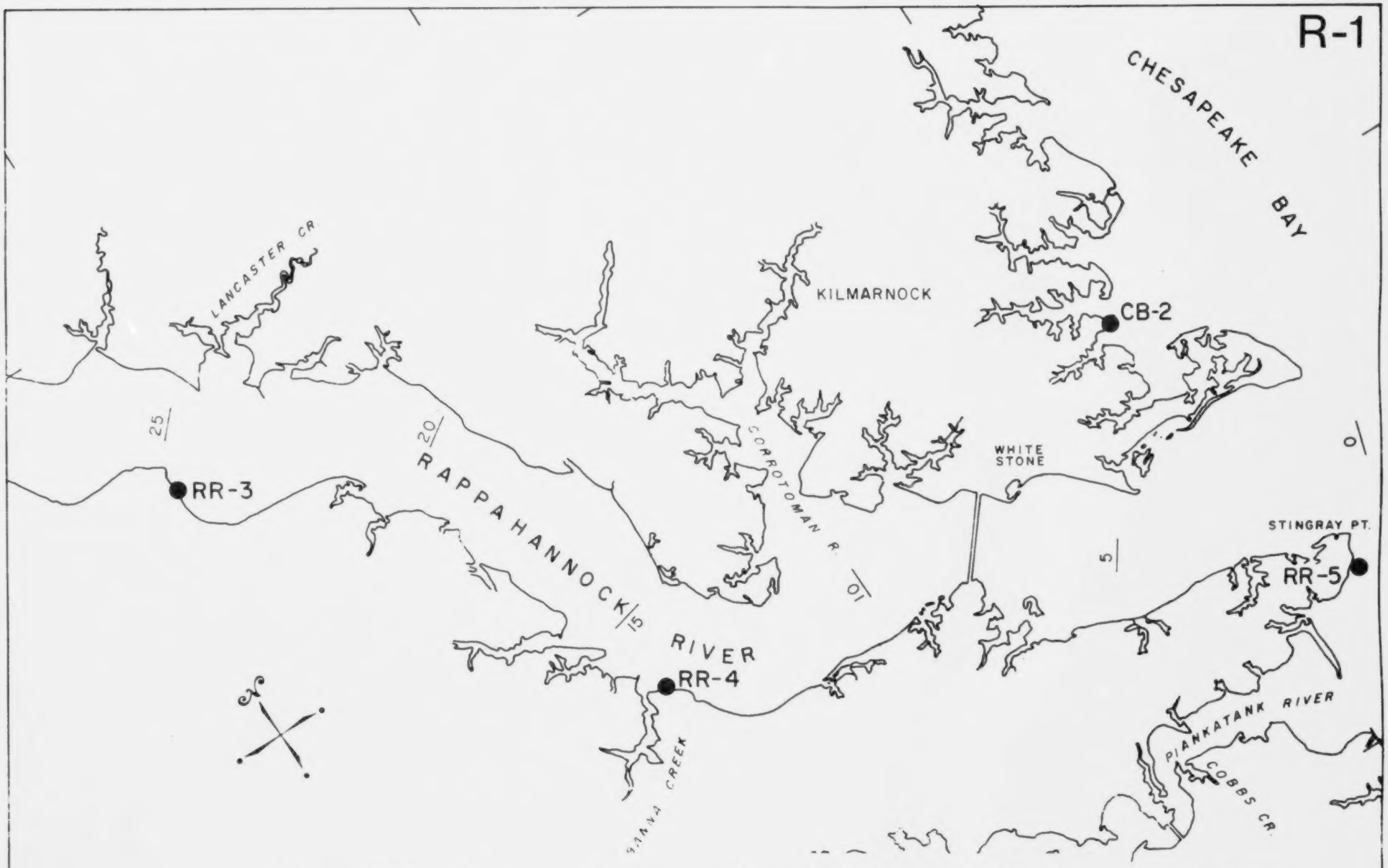
R-1
CHESAPEAKE BAY
KILMARNOCK
CB-2
LANCASTER CR
WHITE STONE
CORROTOMAN R.
STINGRAY PT.
RR-5
RR-3
RAPPAHANNOCK RIVER
RR-4
CREEK
PIANKATANK RIVER
COBBS CR.
MATHEWS
0
5
10
15
20
25

Figure A3.10. Locations of survey stations CB-2 (Fleets Bay), RR-3 (Butylo), RR-4 (Rosegill Farm), and RR-5 (Stingray Point).

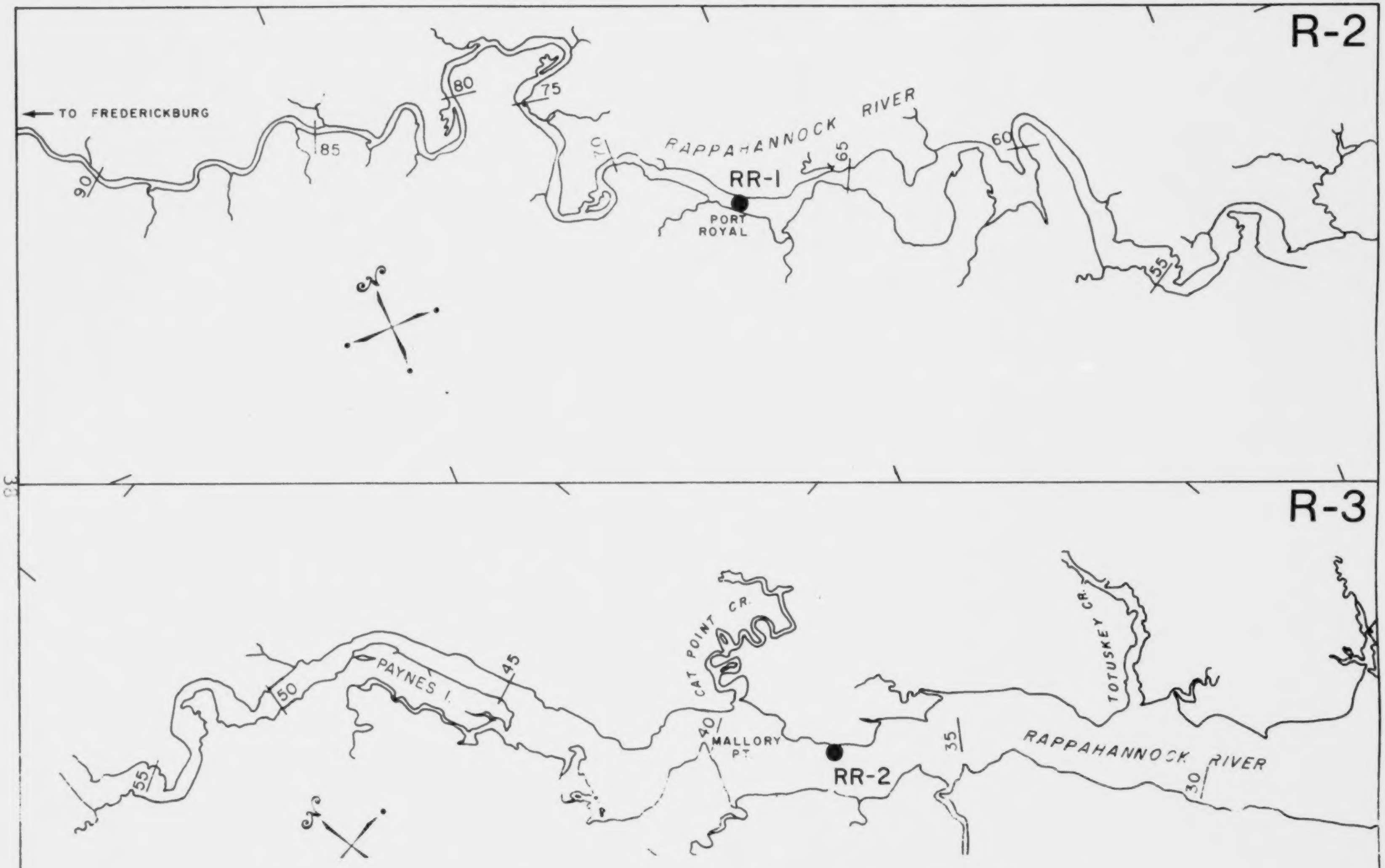
R-2
TO FREDERICKBURG
RAPPAHANNOCK RIVER
RR-1
PORT ROYAL
90
85
80
75
70
65
60
55
R-3
PAYNES I.
CAT POINT CR.
MALLORY PT.
RR-2
TOTUSKEY CR.
RAPPAHANNOCK RIVER
55
50
45
40
35
30

Figure A3.11. Locations of survey stations RR-1 (Port Royal) and RR-2 (Tappahannock).

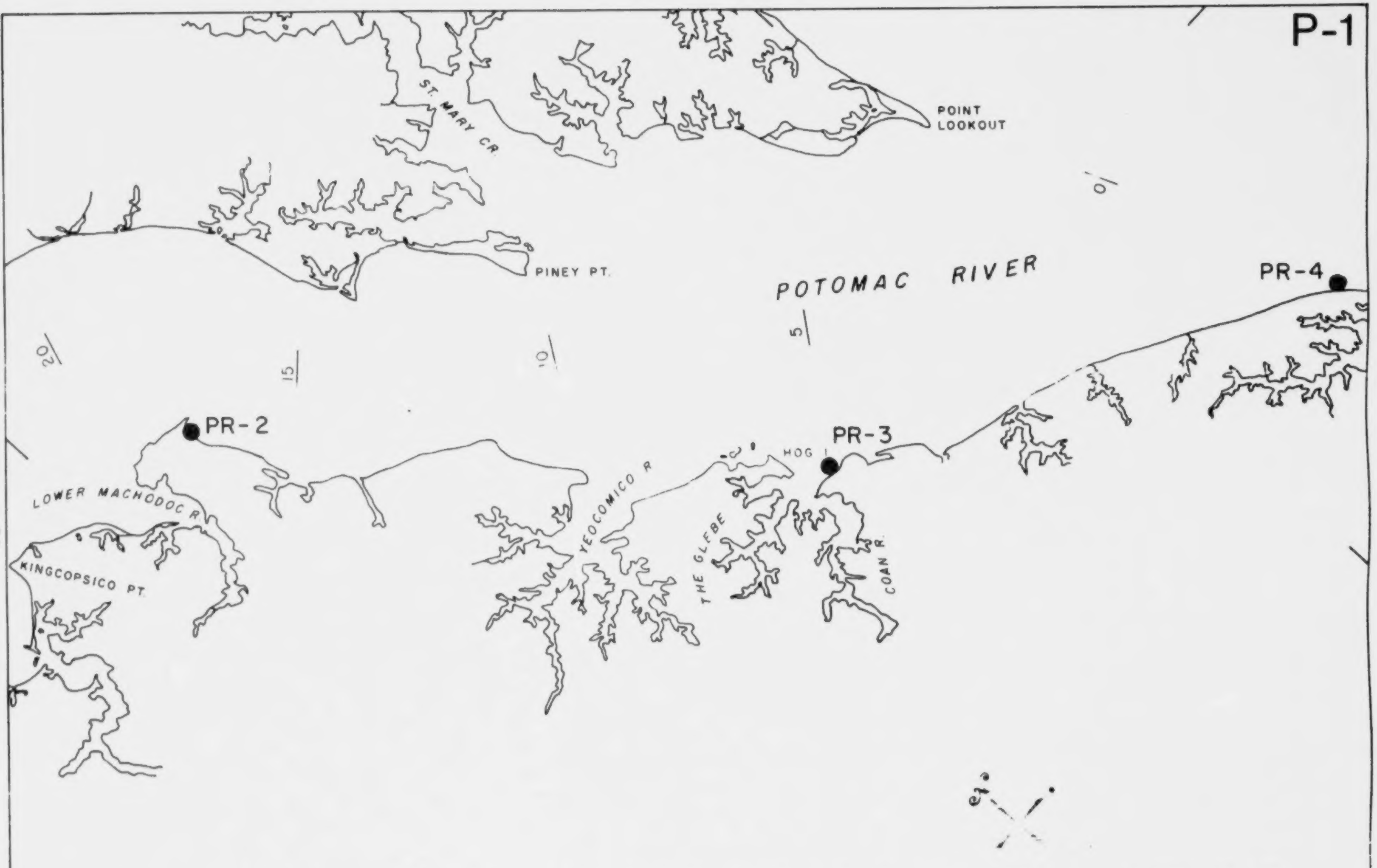
P-1
ST. MARY CR.
POINT LOOKOUT
PINEY PT.
POTOMAC RIVER
PR-4
20
15
10
5
10
PR-2
PR-3
HOG I.
LOWER MACHODOC R.
KINGCOPSICO PT.
YEOCOMICO R.
THE GLEBE
COAN R.

Figure A3.12. Locations of survey stations PR-2 (Ragged Point), PR-3 (Coan River), and PR-4 (Smith Point, Ginny Beach).

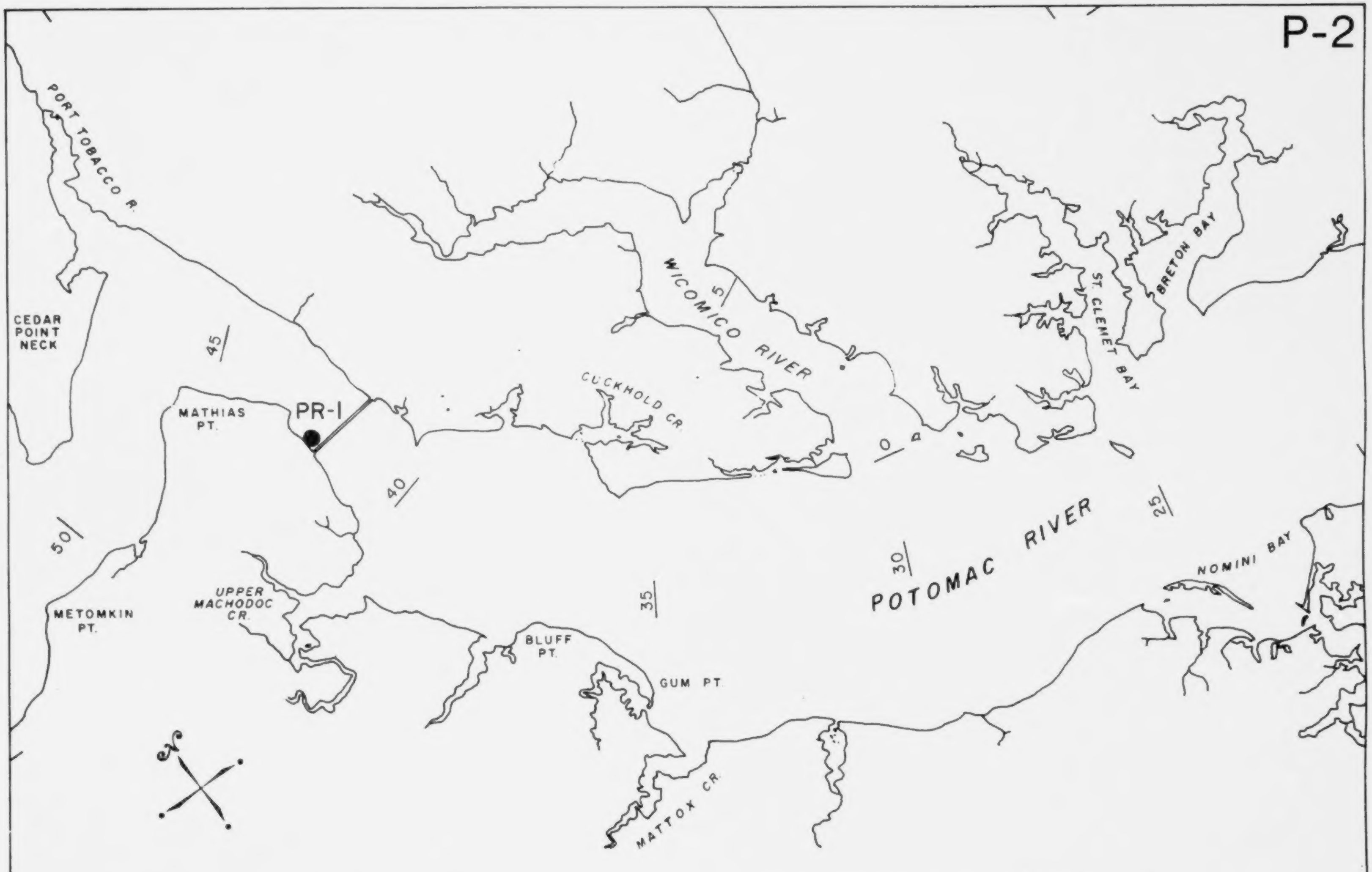
P-2
PORT TOBACCO R.
CEDAR POINT NECK
45
MATHIAS PT.
PR-1
40
50
METOMKIN PT.
UPPER MACHODOC CR.
WICOMICO RIVER
5
CUCKHOLD CR.
0
BLUFF PT.
GUM PT.
MATTOX CR.
35
30
POTOMAC RIVER
25
ST. CLEMET BAY
BRETON BAY
NOMINI BAY

Figure A3.13. Location of survey station PR-1 (Potomac River Bridge).

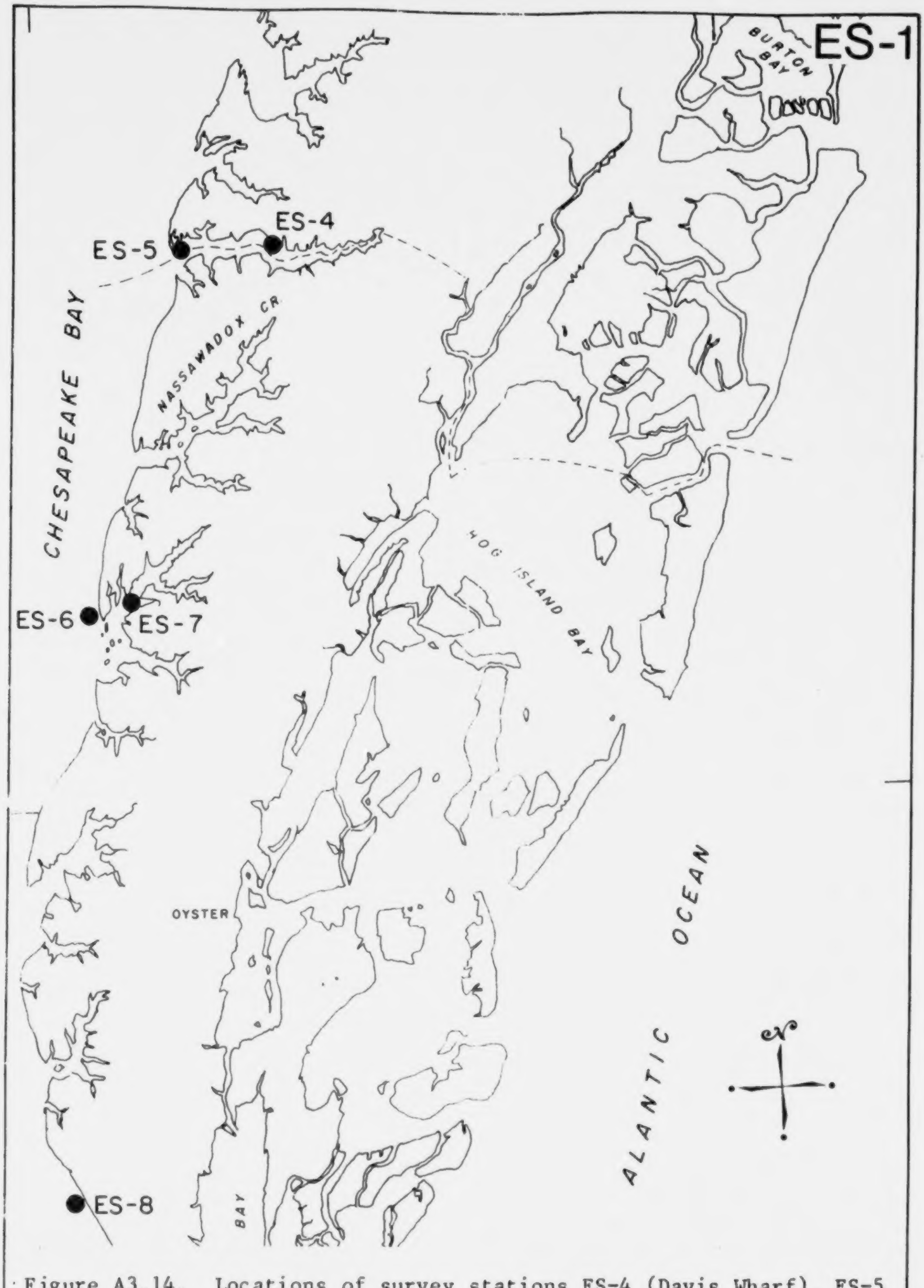
ES-1
BURTON BAY
ES-4
ES-5
CHESAPEAKE BAY
NASSAWADOX CR
HOG ISLAND BAY
ES-6
ES-7
OYSTER
ALANTIC OCEAN
ES-8
BAY
N

Figure A3.14. Locations of survey stations ES-4 (Davis Wharf), ES-5 (Occohannock Creek), ES-6 (Vaucluse Shores), ES-7 (Hungar's Creek) and ES-8 (Picketts Harbor).

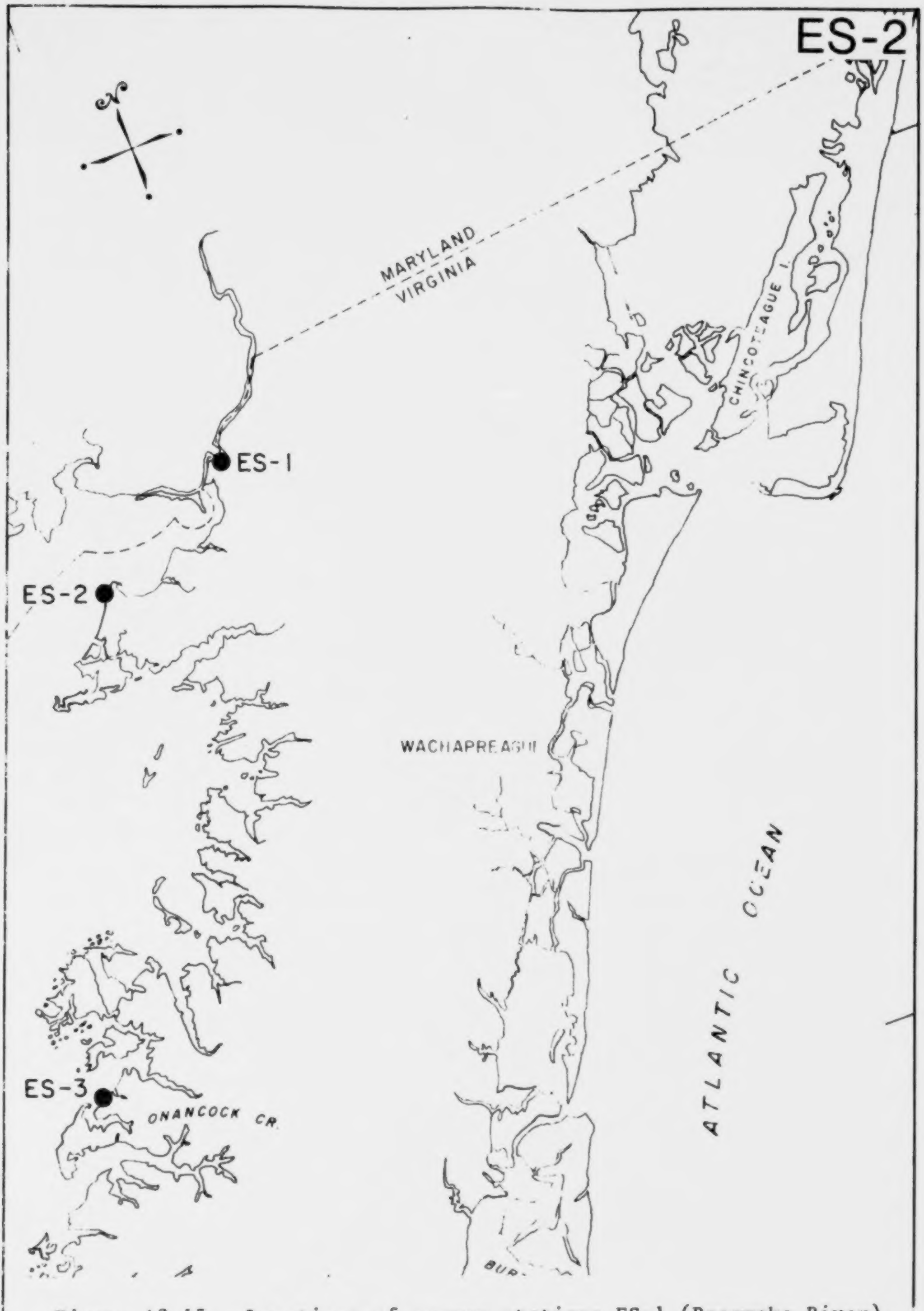
ES-2
N
MARYLAND
VIRGINIA
CHINCOTEAGUE I.
ES-1
ES-2
WACHAPREAGUE
ATLANTIC OCEAN
ES-3
ONANCOCK CR.
BUR

Figure A3.15. Locations of survey stations ES-1 (Pocomoke River), ES-2 (Saxis), and ES-3 (Chesconessex Creek).

SECTION 4

1980 SEVERN RIVER SURVEY PROGRAM

INTRODUCTION

While the 1979 survey of atrazine concentrations in the lower Chesapeake Bay provided evidence of the general level of concentrations, it did not provide evidence of the duration of exposure Zostera beds experienced. To address this question, a survey program was established in the Severn River during 1980. The program involved repeated sampling of the Severn River stations following the first major rainfall after field application of atrazine.

Stations in the Ware River (WR-1 and WR-5) and in the York River (YR-2 and YR-4) were also occupied three times during 1980 to help relate the 1980 data to 1979 survey results.

METHODS

Six stations were occupied in the Severn River during the 1980 survey. They were the same stations occupied during the 1979 survey. Water samples were collected and analyzed as indicated in the analytical methods section of this report (Section 2). Samples were collected at approximately high tide on each sampling date. Sampling was undertaken on April 17 just prior to application of atrazine to fields at the head of the northwest branch of the Severn River. The fields were treated on April 22 and the first rainfall after application occurred two days later, April 24. Sampling began on April 25 and included six collections over an 8 day period. The next major rainstorm occurred on May 18-20. A second set of collections was therefore conducted on May 20, 21, 22 and 23.

Rainfall records were collected from two rain gauges. One is installed at the Virginia Institute of Marine Science at Gloucester Point. It is approximately 10 km south of the fields at the head of the northwest branch of the Severn River. The second rain gauge was situated at Goshen on the Ware River. That gauge is approximately 8 km north of the northwest branch fields.

Estimates of water volume in the northwest branch of the Severn River and estimates of land use acreages in the drainage basin were developed by planimetering areas of USGS topographic maps (Achilles, VA and Clay Bank, VA quadrangles).

RESULTS

The concentrations of atrazine in water samples from the 1980 survey stations are reported in Table 4.1.

The 1980 rainfall records for the gauges at VIMS and on the Ware River are reported in Table 4.2.

The water volumes in the northwest branch of the Severn River and the land use areas in its drainage basin are reported in Table 4.3.

DISCUSSION

The initial sampling on April 17, 1980 found values in the Severn River near or below the detection limits, as did the initial 1979 sampling. The York River station samples were also below detection limits as they were in 1979. The presence of atrazine in the Ware River samples, even at relatively low levels, was unexpected. The values are in excess of any found during the 1979 survey.

The samplings conducted after field application of atrazine and substantial rainfall catalogued the transport of atrazine into the estuary. The rainstorm on the 24th of April delivered approximately one inch of rain to the fields in the northwest branch drainage basin. The day after the rainstorm atrazine was found in detectable amounts at only the two headwater stations. During the following two days concentrations of atrazine decreased in the headwaters and rose to detectable amounts throughout the remainder of the river. Three days after the rainstorm, concentrations in the river were relatively uniform at levels very near the detection limit and well below 1 ppb.

Late on April 27 a second rainstorm moved through the area depositing aproximately one more inch of rain on the fields. This rain event, falling on fields already well saturated, produced rapid and large increases in atrazine concentrations throughout the river. Within 24 hours the station at the river mouth, an area supporting extensive *Zostera* beds attained a 1 ppb level of atrazine in the water. The concentration decreased within two days, despite continued rainfall, to less than 0.3 ppb. Atrazine concentrations in water at upstream stations remained well above 1 ppb for at least four days following the second rainstorm. During that time, however, the concentrations declined to approximately one-fourth those attained immediately after the second rainstorm.

The next major rainfall event occurred in May on the 18th, 19th and 20th. This time atrazine concentrations again rose above the detection limit throughout the river, but they exceeded 1 ppb only at the station in the headwaters.

If the 1980 survey results are taken as representative of long term experience in the Severn River, several observations are important. First, *Zostera marina* beds at the mouth of the river are exposed to levels of atrazine approaching 1 ppb infrequently and only for short periods of time

TABLE 4.1. CONCENTRATIONS OF ATRAZINE IN WATER SAMPLES FROM MOBJACK BAY REGION DURING 1980
(all concentrations in parts per billion)

Station	17 Apr	25 Apr	26 Apr	27 Apr	28 Apr	30 Apr	2 May	20 May	21 May	22 May	23 May
SR-1	≤0.10	6.09	1.29	0.61	16.14	12.60	3.06	0.54	1.10	1.47	1.11
SR-1B	≤0.10	0.15	0.11	0.11	11.48	10.68	2.76	0.40	0.49	0.82	0.75
SR-3A	0.13	≤0.10	0.14	0.12	11.11	9.18	2.15	0.54	0.63	0.74	0.86
SR-4A	≤0.10	≤0.10	0.11	0.11	0.78	1.75	1.20	0.22	0.38	0.39	0.55
SR-5	≤0.10	≤0.10	0.11	0.10	1.42	1.04	0.38	0.30	0.12	0.21	0.39
SR-6	≤0.10	≤0.10	≤0.10	0.12	1.00	0.28	0.22	0.26	≤0.10	≤0.10	0.36
WR-1	0.26	0.11						0.12			
WR-5	0.33	≤0.10						0.12			
YR-2	≤0.10	≤0.10						≤0.10			
YR-4	≤0.10	≤0.10						0.11			

TABLE 4.2. 1980 RAINFALL RECORD FROM GAUGES AT GLOUCESTER POINT (VIMS) AND THE HEAD OF THE WARE RIVER (WARE)

JAN	VIMS	WARE	FEB	VIMS	WARE	MAR	VIMS	WARE	APR	VIMS	WARE	MAY	VIMS	WARE	JUN	VIMS	WARE
1	0	0	1	0	0	1	x	12"	1	0	0	1	0.16	0.13	1	0	0
2	0	0	2	0	0	2	x	snow	2	0	0	2	0	0	2	0	0
3	0	0	3	0	0	3	x	1.0	3	0	0	3	0	0	3	0	0
4	0.30	0.32	4	0	0	4	0	0.24	4	0.11	0.68	4	0	0	4	0	0
5	0.05	0.01	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0
6	0.74	0	6	0	0	6	0	0	6	0	0	6	0.03	0.08	6	0	0.09
7	0	0.01	7	0.20	x	7	0	0	7	0	0	7	0	0	7	1.32	0.18
8	0	0	8	0	x	8	0	0	8	0	0	8	0	0.05	8	0	0.17
9	0	0	9	0.04	x	9	0	0	9	0.34	0.25	9	0	0	9	0	0
10	0	0	10	0.49	x	10	0	0	10	0	0	10	0	0	10	0.42	0.05
11	0.35	0.33	11	0	x	11	0	0.01	11	0	0	11	0	0	11	0.14	0.25
12	0	0	12	0	x	12	0	0	12	0	0	12	0	0	12	0	0
13	0	0	13	0	x	13	0.84	1.73	13	0.49	0.28	13	0	0	13	0	0
14	0.48	0.14	14	0	x	14	0	0	14	0.43	0.40	14	0	0	14	0	0
15	0	0	15	0	x	15	0	0	15	0	0	15	0	0	15	0	0
16	0	0	16	0.66	x	16	0	0	16	0	0	16	0	0	16	0	0.01
17	0	0	17	0	x	17	0	0.24	17	0	0	17	0	0	17	0	0.01
18	0.97	1.06	18	0	x	18	0.52	0.42	18	0	0	18	0.27	0.42	18	0	0
19	0	0	19	0	x	19	0	0	19	0	0	19	x	0.66	19	0	0
20	0	0	20	0	0.01	20	0	0.11	20	0	0	20	x	0.37	20	0	0
21	0	0	21	0	0.01	21	0.98	1.17	21	0	0	21	x	0	21	0	0
22	0.41	0.51	22	0.30	0.40	22	0	0	22	0	0	22	x	0	22	0	0
23	0.43	0.38	23	0.24	0.25	23	0	0	23	0	0.02	23	x	0	23	0	0
24	0	0.02	24	0	0.02	24	0.37	0.58	24	1.34	0.49	24	x	0.08	24	0	0
25	0	0.02	25	0.22	0.28	25	0.13	0	25	0	0.01	25	x	0	25	0	0.02
26	0	0	26	0	0	26	0	0	26	0.04	0.02	26	0	0	26	0	0.06
27	0	0	27	0	0	27	0	0	27	1.42	0.79	27	0	0	27	0	0
28	0	0	28	0	0	28	0.10	0.22	28	0.04	0.34	28	0	0	28	0	0
29	0	0	29	0	0	29	0.33	0.70	29	0.29	0.30	29	0	0	29	0	0
30	0	0				30	0.10	0.17	30	0	0.32	30	0	0.24	30	0	0
31	0	0										31	0	0.01			
TOTAL	3.73	2.80		2.15	0.97		4.50	3.90		4.50	3.90		0.46	2.04		1.88	0.84

(continued)

TABLE 4.2. (continued)

JUL	VIMS	WARE	AUG	VIMS	WARE	SEP	VIMS	WARE	OCT	VIMS	WARE	NOV	VIMS	WARE	DEC	VIMS	WARE
1	0	0	1	0	0	1	0.05	0	1	0.38	0.36	1	0	0	1	0	0
2	0	0	2	0	0	2	0.04	0	2	x	0	2	0	0	2	0	0.02
3	0.14	0.12	3	0.14	0	3	0	0	3	x	0.33	3	0	0	3	0.04	0
4	0	0	4	x	x	4	0	0	4	x	0	4	0.14	0.28	4	0	0
5	0	0.20	5	x	0.01	5	0	x	5	x	0	5	0	0	5	0	0
6	x	0	6	x	0	6	0	x	6	0	0.02	6	0	0	6	0	0
7	0	0	7	x	0	7	0.03	0.01	7	0	0	7	0	0	7	0	0
8	0.22	0.38	8	x	0	8	0	0	8	0	0	8	0	0	8	0	0
9	0.04	0.10	9	x	0	9	0	0	9	0	0	9	0.05	0.17	9	0	0
10	0.08	0	10	x	0	10	0.14	0.27	10	0	0.01	10	0	0	10	0.42	0.38
11	0	0	11	0	0	11	0	0	11	0.54	0.75	11	0	0	11	0	0
12	0.40	0.86	12	0	0.01	12	0	0	12	0	0	12	0	0	12	0	0
13	0	0.01	13	0	0	13	0	0	13	0	0	13	0	0	13	0	0
14	0	0	14	0	0	14	0	0	14	0	0	14	0	0	14	0	0
15	0	0	15	0.20	0	15	0	0	15	0	0	15	0	0.04	15	0	0
16	0	0	16	0.19	0	16	0	0	16	0	0	16	0	0.06	16	0.05	0.06
17	1.31	0.89	17	0	0	17	0.08	0	17	0	0	17	0.76	1.05	17	0	0
18	0	0	18	0.19	0.14	18	0	0	18	0	0	18	0	0.11	18	0	0
19	0	0	19	0	0	19	0	0	19	0.74	1.15	19	0	0	19	0	0
20	0	0	20	0	0.01	20	0	0	20	0	0	20	0	0	20	0	0
21	0	0	21	0	0	21	0	0	21	0	0	21	0.04	0	21	0	0
22	0	0	22	0	0	22	0	0	22	0	0	22	0	0	22	0	0
23	0.65	0.96	23	0	0	23	0.05	0	23	0	0	23	0	0.05	23	0.39	0.50
24	0	0	24	0	0	24	0	0	24	0.15	0.20	24	0.83	1.01	24	0.03	0.04
25	0	0	25	0	0	25	1.10	2.09	25	1.22	2.13	25	0	0	25	0	0
26	0	0	26	0	0	26	0	0	26	0	0	26	0	0	26	0.10	0
27	0	0	27	0	0	27	0	0	27	0	0	27	0.14	0.16	27	0.05	0.09
28	0	0	28	0	0	28	0	0	28	0.04	0.05	28	0	0.02	28	0.03	0.14
29	0.02	0.05	29	0	0	29	0	0	29	0.01	0.01	29	0	0	29	0.15	0.20
30	0	0	30	0	x	30	0.09	0.14	30	0.34	0.33	30	0	0	30	0	0
31	0	0	31	0.31	x				31	0	0				31	0	0
TOTAL	2.86	3.57		1.03	0.52		1.58	2.51		3.42	5.52		1.96	2.95		1.26	1.44

TABLE 4.3. NORTHWEST BRANCH OF SEVERN RIVER

total drainage basin = 35.54 km^2

open water = 4.36 km^2

intertidal marsh = 0.68 km^2

pasture and residential area = 6.33 km^2

cropland area = 5.57 km^2

forested area = 18.60 km^2

branch mean low water volume = 7.01 x 10^9 liters

tidal volume = 1.96 x 10^9 liters

branch mean high water volume = 8.97 x 10^9 liters (less than two days). If grass beds once grew throughout the lower one-half of the river system, the upstream beds may have occasionally been exposed to atrazine concentrations exceeding 1 ppb, but much less than 10 ppb. The exposures, however, were probably limited to spring and probably did not last for more than a week or two.

In general, the data collected in this survey program suggest that Zostera marina, in its present distribution, is unlikely to experience levels of atrazine in water in excess of 1 ppb for more than one to two days during a growing season. This conclusion requires several major assumptions. The first assumption is that the Severn River is a good model for the lower Chesapeake Bay system. The second assumption is that the spring runoff event monitored in 1980 is an unusual event and fairly represents a "worst case" situation.

The latter assumption is probably a reasonable one. The combination of two major rainstorms dropping approximately two inches of rain within several days of field application of atrazine is unusual. A review of daily rainfall from a number of recording stations in the region (U.S. Environmental Data Service) indicate that during the eleven year period 1971 through 1981, a greater amount of rain has fallen in late April on only two occasions. An average and median amount over the twelve year period was approximately 1.2 inches of rainfall. Data from the Williamsburg, Virginia, station is summarized in the appendix to this section as an example (Table B4.1).

The first assumption that the Severn River is a good model for lower Chesapeake Bay systems is more tenuous. The 1979 survey program suggests the Severn River stations, despite a limited geographic range, experience atrazine loadings which cover the entire range of exposures in the lower Bay. Furthermore, the general trend of concentations from headwaters to river mouth seems typical of the other river systems sampled in 1979. The general pattern of land use in the Severn River is not unusual for the larger rivers, particularly in respect to the proportion of croplands. The topography of the drainage basin is atypically flat, but this factor is somewhat compensated by the proportionately reduced scale of the entire system. The major difficulty with using the Severn River as a model for other Bay systems is the lack of specific information about circulation within the river. In the absence of information about water parcel residence times in the river, particularly during runoff events, extrapolation of herbicide exposures to other systems must remain intuitive.

The principal objective of the 1980 survey program was to evaluate the duration of Zostera marina exposures to atrazine during a growing season. Building on data collected during the 1979 survey, a program was instituted to monitor the spring runoff events in the Severn River. Fortunately for this effort, there was an unusual amount of rain immediately after field applications of atrazine in the Severn drainage basin. The monitoring program determined that existing grass beds within the Severn River were exposed to atrazine concentrations as high as 1 ppb for a period of less than two days. Reaches of the river which may have contained Zostera beds in the past, were exposed to water concentrations of atrazine in excess of 1 ppb, but less than 10 ppb, for a period of approximately one week.

In general, the 1980 survey program is presumed to indicate that Zostera marina, in its present distribution, rarely is exposed to atrazine concentrations in excess of 1 ppb.

REFERENCES

U.S. Environmental Data Service. Climatological Data-Virginia. Volumes 81-91. Published by U.S. Department of Commerce.

TABLE B4.1. SUMMARY OF ELEVEN YEAR RAINFALL DATA FOR WILLIAMSBURG, VIRGINIA, GAUGING STATION

Year	Total April Rainfall (in.)	Amount During Last 15 Days in April
1971	1.71	0.52
1972	3.80	2.29
1973	3.42	1.61
1974	1.48	0.34
1975	3.19	0.99
1976	0.77	0.00
1977	3.87	1.29
1978	4.20	4.06
1979	3.88	1.16
1980	3.05	1.62
1981	2.62	1.14
Average	2.91	1.37
Median	3.19	1.16

SECTION 5

FIELD DOSING STUDIES

INTRODUCTION

Short term effects of atrazine on the *Zostera marina* community were investigated by *in situ* dosing experiments. Since the mode of action of atrazine is to block the Hill reaction in photosynthesis, the short term studies were designed to measure changes in oxygen production. Oxygen production was also selected as a test parameter to provide correlations with the data sets being generated by Wetzel *et al*. (1979) as part of their studies of production in the *Zostera* community.

The concentrations of atrazine selected for study ranged up to and including 1000 ppb. The survey program results suggested the 1 ppb and 10 ppb concentrations should be of greatest interest since they represent the range of values actually found in Bay waters. Higher concentrations were included to ensure detection of an effect on oxygen production.

Some of the field dosing experiments were designed to test the hypothesis that atrazine acts in an additive or synergistic fashion with reduced light levels to produce significant impacts on the *Zostera* community. The hypothesis was suggested by the high probability of co-occurrence of maximum atrazine concentrations and high turbidity during post planting spring runoff events. The hypothesis was tested by adding greenhouse shading material to some of the dosing enclosures. Effective insolation was thus reduced to 80%, 70% or 50% of natural conditions.

METHODS

In situ dosing of the *Zostera marina* community was accomplished with hemispherical plexiglass enclosures. The enclosures are identical to those used by Wetzel *et al* (1982). Each dome enclosure has a volume of approximately 260 liters. Six domes were generally used simultaneously to provide control and atrazine treatments.

Before "setting" the domes, the working platform was positioned and anchored. The grass bed in the vicinity of the platform stern was surveyed for uniformity by divers. Each of the six domes was carefully positioned on the bottom and the four inch vertical flange on the perimeter of the dome was driven into the sediment. This provided a "seal" effectively isolating the dome contents from the surrounding system. Ambient water was then pumped through the dome to flush it for approximately one hour. An experiment was initiated by closing all portals in the dome, so that a closed loop between the dome and an onboard pumping station was created. Atrazine was introduced to the closed system through septa in the apex of the dome. Atrazine standards, prepared in the laboratory, were injected by 50 ml glass syringes. The standards consisted of technical grade atrazine dissolved in 100 ml methanol (for 1 ppb through 100 ppb atrazine treatments) or 200 ml methanol (for 1000 ppb atrazine treatment).

The amount of atrazine was selected to give the appropriate nominal concentration of atrazine in water. Methanol controls were run in the first several experiments to identify effects due to the atrazine carrier.

Domes were usually "set" at or near sunrise. Experiments typically lasted until near sunset. Dissolved oxygen in the domes was monitored hourly by inserting an oxygen meter probe (YSI or Orbisphere) into a port on the pumping station. Near termination of the experiment water samples were taken for atrazine analysis (500 ml). Samples were also collected for dissolved oxygen determination by Winkler titration as a check on the oxygen meters.

Shading experiments were conducted by making individual shades for domes. The shades were constructed of greenhouse shade cloth (a coarse woven nylon material). Insolation is controlled by coarseness of the weave in the material. For these experiments the material used blocked 20%, 30% or 50% of incident light without altering the spectrum of the transmitted light.

RESULTS

Data from the experiments are presented in the appendix to this section (Appendix C). Tables C5.1 through C5.18 present the hourly dissolved oxygen concentrations (in ppm). Figures C5.1 through C5.18 are graphs of the oxygen concentrations versus time. Tables C5.19 through C5.36 contain the calculated oxygen production rates (in mg O_2 m^{-2} hr^{-1}) for each experiment.

For analytical purposes each experiment was divided into five time periods based on the sun's declination (morning 0700-1000, noon 1100-1400, afternoon 1500-1800, evening 1900-2300, night 0000-0600). Within each time period the oxygen production values for each treatment were averaged and the mean values compared using the F Test. Table 5.1 presents the results of the analyses for each period of each experiment.

Experiments with significant differences between the mean rates of oxygen production were further analyzed by the multiple range test in order to indicate probable associations among the treatments. Table 5.2 presents the results of these analyses.

Table 5.3 presents analyses of water samples collected from the domes at the conclusion of dosing. The samples were taken as a check on the nominal concentrations assumed for each treatment.

DISCUSSION

Review of the field dosing experiments indicates consistent and significant negative effects of atrazine dosing were only detected at the

TABLE 5.1. STATISTICAL EVALUATION OF GUINEA MARSH DOME STUDY DATA USING 5% LEVEL F TESTS TO TEST DIFFERENCES BETWEEN MEAN OXYGEN PRODUCTION RATES FOR EACH EXPERIMENTAL PERIOD

Exp.	Period	Date	F Ratio	F Probability	Significant at .05 Level
5.1	afternoon	5-29-80	1.004	0.4777	-
5.1	evening	5-29-80	1.279	0.3154	-
5.1	night	5-29-80	0.399	0.7553	-
5.1	morning	5-30-80	2.184	0.1428	-
5.1	noon	5-30-80	1.235	0.3399	-
5.1	afternoon	5-30-80	0.221	0.8776	-
5.2	morning	6-23-80	1.721	0.2156	-
5.2	noon	6-23-80	2.906	0.0784	-
5.2	afternoon	6-23-80	0.003	0.9997	-
5.2	evening	6-23-80	2.723	0.0909	-
5.2	night	5-23-80	1.130	0.3609	-
5.3	morning	6-25-80	5.047	0.0101	*
5.3	noon	6-25-80	2.414	0.0766	-
5.3	afternoon	6-25-80	1.239	0.3318	-
5.3	evening	6-25-80	3.421	0.0272	*
5.3	night	6-25-80	0.467	0.7993	-
5.5	noon	7-14-80	17.272	0.0000	*
5.5	noon	7-14-80	8.703	0.0002	*
5.5	afternoon	7-14-80	2.061	0.1415	-
5.4	morning	7-15-80	1.672	0.1924	-
5.4	noon	7-15-80	15.876	0.0000	*
5.4	afternoon	7-15-80	0.324	0.8915	-
5.4	evening	7-15-80	40.525	0.0000	*
5.4	night	7-15-80	2.937	0.0252	*
5.4	morning	7-16-80	38.735	0.0000	*
5.4	noon	7-16-80	84.010	0.0000	*
5.6	morning	7-18-80	12.826	0.0000	*
5.6	noon	7-18-80	12.380	0.0000	*
5.6	afternoon	7-18-80	0.754	0.5942	-
5.6	evening	7-18-80	6.318	0.0007	*
5.6	night	7-18-80	1.598	0.1909	-
5.7	morning	7-29-80	0.506	0.7683	-
5.7	noon	7-29-80	3.583	0.0201	*
5.7	afternoon	7-29-80	1.187	0.3715	-
5.7	evening	7-29-80	3.305	0.0416	*
5.8	morning	7-30-80	5.519	0.0072	*
5.8	noon	7-30-80	2.429	0.0753	-
5.8	afternoon	7-30-80	0.244	0.9375	-

(continued)

TABLE 5.1. (continued)

Exp.	Period	Date	F Ratio	F Probability	Significant at .05 Level
5.9	morning	7-31-80	8.821	0.0010	*
5.9	noon	7-31-80	0.094	0.9921	-
5.9	afternoon	7-31-80	0.109	0.9884	-
5.10	morning	8-1-80	6.669	0.0144	*
5.10	noon	8-1-80	34.920	0.0000	*
5.11	morning	8-12-80	0.664	0.6652	-
5.11	noon	8-12-80	7.782	0.0005	*
5.11	afternoon	8-12-80	0.093	0.9925	-
5.12	morning	8-13-80	0.945	0.4871	-
5.12	noon	8-13-80	3.289	0.0277	*
5.12	afternoon	8-13-80	0.124	0.9852	-
5.13	morning	8-14-80	1.934	0.1620	-
5.13	noon	8-14-80	0.259	0.9294	-
5.13	afternoon	8-14-80	0.763	0.5906	-
5.13	evening	8-14-80	2.035	0.1355	-
5.14	morning	8-15-80	1.935	0.1619	-
5.14	noon	8-15-80	6.336	0.0017	*
5.14	afternoon	8-15-80	1.049	0.4683	-
5.15	morning	9-8-80	1.345	0.3601	-
5.15	noon	9-8-80	3.775	0.0163	*
5.15	afternoon	9-8-80	0.203	0.9570	-
5.16	morning	9-9-80	0.434	0.8168	-
5.16	noon	9-9-80	4.010	0.0128	*
5.16	afternoon	9-9-80	0.488	0.7761	-
5.17	morning	9-10-80	0.309	0.8984	-
5.17	noon	9-10-80	0.760	0.590	-
5.17	afternoon	9-10-80	0.657	0.6605	-
5.18	morning	9-11-80	1.171	0.3782	-
5.18	noon	9-11-80	2.479	0.0709	-
5.18	afternoon	9-11-80	0.143	0.9784	-

*Morning	0700-1000
Noon	1100-1400
Afternoon	1500-1800
Evening	1900-2300
Night	0000-0600

TABLE 5.2. STATISTICAL EVALUATION OF GUINEA MARSH DOME STUDY DATA USING 5% LEVEL STUDENT-NEWMAN-KEULS MULTIPLE RANGE TEST

Experiment/ Period	Atrazine Concentration*	Shading Level	Results of S-N-K 5% Level		
5.3 morning	1000 ppb	control	*		
	100 ppb	control	*	*	
	1 ppb	control		*	
	10 ppb	control		*	
	MEOH control	control		*	
	control	control		*	
5.3 evening	MEOH control	control	*		
	control	control	*		
	10 ppb	control	*		
	1 ppb	control	*		
	100 ppb	control	*		
	1000 ppb	control	*		
5.4 noon (7/15/80)	1000 ppb	control	*		
	100 ppb	control		*	
	1 ppb	control			*
	10 ppb	control			*
	MEOH control	control			*
	control	control			*
5.4 evening (7/15/80)	control	control	*		
	10 ppb	control	*		
	1 ppb	control	*	*	
	MEOH control	control	*	*	
	100 ppb	control		*	
	1000 ppb	control			*
5.4 night (7/15/80)	MEOH control	control	*		
	1 ppb	control	*		
	10 ppb	control	*		
	control	control	*		
	100 ppb	control	*		
	1000 ppb	control	*		

(continued)

TABLE 5.2. (continued)

Experiment/ Period	Atrazine Concentration*	Shading Level	Results of S-N-K 5% Level			
5.4 morning (7/16/80)	1000 ppb	control	*			
	100 ppb	control	*			
	10 ppb	control		*		
	1 ppb	control			*	
	MEOH control	control			*	*
	control	control				*
5.4 noon (7/16/80)	100 ppb	control	*			
	1000 ppb	control	*			
	10 ppb	control		*		
	1 ppb	control			*	
	MEOH control	control			*	
	control	control				*
5.5 morning	1000 ppb	control	*			
	100 ppb	control		*		
	control	control		*		
	MEOH control	control		*		
	1 ppb	control		*		
	10 ppb	control		*		
5.5 noon	1000 ppb	control	*			
	MEOH control	control		*		
	100 ppb	control		*		
	control	control		*		
	1 ppb	control		*		
	10 ppb	control		*		
5.6 morning	1000 ppb	control	*			
	10 ppb	control		*		
	100 ppb	control		*		
	control	control		*		
	1 ppb	control		*		
	MEOH control	control		*		
5.6 noon	1000 ppb	control	*			
	100 ppb	control		*		
	10 ppb	control		*	*	
	1 ppb	control			*	
	MEOH control	control			*	
	control	control			*	

(continued)

TABLE 5.2. (continued)

Experiment/ Period	Atrazine Concentration*	Shading Level	Results of S-N-K 5% Level		
5.6 evening	1 ppb	control	*		
	MEOH control	control	*		
	control	control	*		
	10 ppb	control	*	*	
	100 ppb	control	*	*	
	1000 ppb	control		*	
5.7 noon	10 ppb	51%	*		
	MEOH control	51%	*		
	control	51%	*		
	10 ppb	control	*		
	MEOH control	control	*		
	control	control	*		
5.7 evening	control	control	*		
	MEOH control	control	*		
	10 ppb	control	*		
	control	51%	*		
	10 ppb	51%	*		
	MEOH control	51%	*		
5.8 morning	MEOH control	51%	*		
	control	51%	*		
	10 ppb	51%	*		
	10 ppb	control	*	*	
	control	control	*	*	
	MEOH control	control	*	*	
5.9 morning	MEOH control	51%	*		
	10 ppb	51%	*	*	
	10 ppb	control	*	*	
	control	control	*	*	
	MEOH control	control		*	
	control	51%			*
5.10 morning	10 ppb	30%	*		
	control	30%	*	*	
	control	control		*	
	10 ppb	control		*	
5.10 noon	10 ppb	30%	*		
	control	30%		*	
	10 ppb	control		*	
	control	control			*

(continued)

TABLE 5.2. (continued)

Experiment/ Period	Atrazine Concentration*	Shading Level	Results of S-N-K 5% Level	
5.11 noon	control	30%	*	
	10 ppb	30%	*	
	1 ppb	30%	*	
	control	control		*
	10 ppb	control		*
	1 ppb	control		*
5.12 noon	10 ppb	30%	*	
	control	30%	*	
	1 ppb	30%	*	
	control	control	*	
	10 ppb	control	*	
	1 ppb	control	*	
5.14 noon	1 ppb	control	*	
	10 ppb	30%	*	
	10 ppb	control	*	
	1 ppb	30%	*	
	control	30%	*	
	control	control		*
5.15 noon	1 ppb	20%	*	
	control	20%	*	
	10 ppb	20%	*	*
	1 ppb	control	*	*
	control	control	*	*
	10 ppb	control		*
5.16 noon	10 ppb	20%	*	
	1 ppb	20%	*	
	control	20%	*	
	10 ppb	control	*	
	control	control	*	
	1 ppb	control	*	

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* Atrazine concentrations are ranked in order of lowest mean productivity rates (mg O_2 m^{-2} hr^{-1}) to highest mean productivity rates.

TABLE 5.3. ATRAZINE CONCENTRATION IN WATER, NOMINAL VS MEASURED CONCENTRATIONS
(samples generally taken at termination of experiment)

Experiment	Date	Treatment Nom. Conc. & % Shade	Measured Concentration
5.1	5-29-80	control	
		MeOH control	
		100 ppb	
		100 ppb - 100%	
5.2	6-23-80	control	0.16 ppb
		MeOH control	0.11 ppb
		10 ppb	2.54 ppb
		100 ppb	65.15 ppb
5.3	6-25-80	control	0.28 ppb
		MeOH control	0.10 ppb
		1 ppb	1.48 ppb
		10 ppb	6.14 ppb
		100 ppb	72.49 ppb
		1000 ppb	515.15 ppb
5.4	7-15-80	control	0.16 ppb
		MeOH control	0.17 ppb
		1 ppb	1.47 ppb
		10 ppb	6.38 ppb
		100 ppb	71.85 ppb
		1000 ppb	761.90 ppb
5.5	7-14-80	control	
		MeOH control	
		1 ppb	
		10 ppb	
		100 ppb	
		1000 ppb	
5.6	7-18-80	control	0.21 ppb
		MeOH control	≤0.10 ppb
		1 ppb	0.81 ppb
		10 ppb	4.85 ppb
		100 ppb	61.44 ppb
		1000 ppb	709.29 ppb
5.7	7-29-80	control	0.24 ppb
		control-51%	0.24 ppb
		MeOH control	0.58 ppb
		MeOH-51%	0.58 ppb
		10 ppb	6.51 ppb
		10 ppb 51%	9.87 ppb

(continued)

TABLE 5.3. (continued)

Experiment	Date	Treatment Nom. Conc. & % Shade	Measured Concentration
5.8	7-30-80	control	0.18 ppb
		control 51%	no sample
		MeOH control	no sample
		MeOH - 51%	no sample
		10 ppb	6.30 ppb
		10 ppb - 51%	7.86 ppb
5.9	7-31-80	control	
		control -51%	
		MeOH control	
		MeOH - 51%	
		10 ppb	6.51 ppb
		10 ppb - 51%	8.81 ppb
5.10	8-1-80	control	
		control - 30%	
		10 ppb	
		10 ppb - 30%	
5.11	8-12-80	control	
		control - 30%	
		1 ppb	0.63 ppb
		1 ppb - 30%	0.36 ppb
		10 ppb	7.41 ppb
		10 ppb - 30%	6.93 ppb
5.12	8-13-80	control	
		control - 30%	
		1 ppb	0.72 ppb
		1 ppb - 30%	0.69 ppb
		10 ppb	6.58 ppb
		10 ppb - 30%	6.35 ppb
5.13	8-14-80	control	
		control - 30%	
		1 ppb	1.02 ppb
		1 ppb - 30%	0.85 ppb
		10 ppb	7.18 ppb
		10 ppb - 30%	6.54 ppb
5.14	8-15-80	control	
		control - 30%	
		1 ppb	
		1 ppb - 30%	
		10 ppb	
		10 ppb - 30%	

(continued)

TABLE 5.3. (continued)

Experiment	Date	Treatment Nom. Conc. & % Shade	Measured Concentration
5.15	9-8-80	control	
		control - 20%	0.13 ppb
		1 ppb	
		1 ppb - 20%	0.60 ppb
		10 ppb	
		10 ppb - 20%	6.59 ppb
5.16	9-9-80	control	
		control - 20%	
		1 ppb	
		10 ppb	
		10 ppb - 20%	
5.17	9-10-80	control	
		control - 20%	≤0.10 ppb
		1 ppb	
		1 ppb - 20%	0.26 ppb
		10 ppb	
		10 ppb - 20%	6.79 ppb
5.18	9-11-80	control	
		control - 20%	≤0.10 ppb
		1 ppb	
		1 ppb - 20%	0.59 ppb
		10 ppb	
		10 ppb - 20%	7.77 ppb

highest concentration, 1000 ppb. Productivity, as measured by oxygen production, was frequently reduced by 100 ppb atrazine concentrations but the difference from controls was not always statistically significant as determined by multiple range testing. The data for lower concentrations of atrazine was even more variable, preventing significant conclusions about effects. A priori expectations were for a graded response of oxygen production reduction positively correlated with atrazine concentration. A number of the experiments produced results which fit these expectations (see Figure C5.6 for example) however, we have found no basis in any of the information we collected for conclusions based only on selected experiments. We felt constrained therefore to analysis of the entire data set and caution against any selective interpretations.

The in situ enclosure techniques proved unable to distinguish moderate effects of atrazine from control responses. The principal reason for this appears to be the natural variability of the Zostera community. Despite our efforts to obtain a homogeneous set of enclosed communities for each experiment we were obviously unable to achieve a reduction in variation sufficient to permit statistically significant detection of anything other than major effects. Detailed sampling of the enclosed communities in each experiment may have permitted better resolution of the data, but unfortunately suitable data was not collected during these studies.

The shading experiments generally produced the expected reduction in production, but no statistically significant evidence of either additive or synergistic effects with atrazine dosing was developed.

The results of analysis of the water samples collected from the domes revealed a persistent sub-part-per billion level of atrazine within the control domes. The results are not due to analytical errors. Great care was exercised in the field to minimize any chances for cross-contamination. Specific sets of experimental gear were routinely used for the control and dosed treatments and each enclosure was run as a closed system throughout the experiments. Ambient atrazine concentrations were always below our detection limits at the experimental site. Nevertheless, low-level contamination of the controls remained a persistent problem.

The water samples also indicate a fairly consistent recovery of 60-70% of the injected spike at the conclusion of each experiment. Attempts were made to investigate partitioning of the atrazine spike among water, sediments, plants and epiphytes within the domes during the course of the experiments. Satisfactory sampling methods proved to be an intractable problem. Despite several attempts to collect usable samples of each substrate we had not solved the methodology problem by the conclusion of this project.

In summary, the results of the field dosing experiments appear to be limited by the methodology. The finding that atrazine concentrations of 100 ppb and greater generally produced a significant effect on short-term net productivity of the Zostera community is in general agreement with the results of the greenhouse dosing studies reported in the following section. Conclusions about effects of lower concentrations of atrazine on Zostera communities, either the presence or absence of effects, are generally not supported by the data generated in this investigation.

A more intensive use of the *in situ* enclosure methodology may permit better definition of effects in the future. Specifically, greater replication of both control and low-level doses will be required. Much of the current data may have been more useful if information about the enclosed community (e.g. macrophyte and epiphyte biomasses) has been available. This information would permit efforts to normalize the observed oxygen production effects, factoring out nonhomogeneity of the enclosed communities. From our experience, development of this information requires a major commitment of resources (see also Orth et al. 1982). As indicated by the analytical problems we have had, however, the commitment is essential.

Questions raised by this study which remain unanswered include description of the partitioning of atrazine among components of the enclosed *Zostera* community, and analysis of the response of *Zostera* to other forms of atrazine exposure (e.g. atrazine sorbed to suspended sediments). Both of these questions are important to efforts to extrapolate this type of experimental data to natural communities.

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TABLE C5.1. DOME STUDY, 29-30 MAY 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

Time	Temp.	Ambient DO	Dome 7	Dome 8	Dome 9	Dome 10
1800	25°C	7.9	8.4	8.5	8.2	7.9
1900	25°	7.7	7.8	7.9	7.8	6.8
2000	23°	7.2	6.7	6.9	7.1	6.2
2100	22.5°	7.6	6.0	5.9	6.5	5.4
2200	23°	6.6	4.8	4.2	5.3	4.3
2300	23°	5.5	5.3	3.9	4.8	3.8
2400	23°	6.3	4.4	2.2	4.2	2.9
0100	24°	5.3	4.0	1.2	3.0	2.6
0200	24°	5.6	3.4	1.4	3.0	2.0
0300	23°	4.8	2.6	0.6	1.8	0.8
0400	*					
0500	23°	4.6	1.8	0.1	1.3	0.6
0600	22.5°	5.0	2.6	0.6	1.6	0.9
0700	22°	5.4	2.4	0.2	1.0	0.3
0800	22°	5.6	3.0	0.3	0.6	0.0
0900	22°	5.4	3.5	0.2	0.2	0.0
1000	22°	5.5	4.0	0.8	0.7	0.0
1100	23°	4.8	5.6	2.0	1.1	0.2
1200**	24°	5.6	6.2	3.0	1.6	0.4
1300	24.5°	5.9	6.4	3.2	1.4	0.0
1400	25°	9.0	10.9	6.5	3.0	0.2
1500	25°	9.0	11.5	7.4	3.3	0.2
1600	26°	8.8	12.0	8.4	3.6	0.2
1700	25.5°	11.1	12.6	9.0	3.8	0.2
1800	25.5°	11.5	12.6	9.0	3.9	0.4

dome 7 - control
dome 8 - 100 ml MeOH
dome 9 - 100 ppb atrazine
dome 10- 100 ppb atrazine

*no readings taken
**Probe malfunction-membrane replaced after 1300 reading

TABLE C5.2. DOME STUDY, 23-24 JUNE 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

Time	Ambient Temp.	DO	Dome 5	Dome 7	Dome 8	Dome 10
0800	22°C	6.1	5.9	5.7	6.4	5.4
0900	22°	6.4	6.3	6.3	7.0	5.3
1000	22.5°	6.9	7.1	7.4	7.6	6.1
1100	23°	7.7	9.4	10.0	9.3	6.2
1200	23.5°	8.0	10.0	10.8	10.3	6.2
1300	24°	8.6	10.8	12.0	11.3	6.2
1400	25°	9.4	11.4	12.8	12.0	6.0
1500	25°	9.4	11.5	13.1	12.3	6.1
1600	25.5°	8.4	11.7	13.2	12.4	5.8
1700	25.5°	8.0	11.5	12.8	11.6	4.6
1800	25°	7.4	10.6	11.9	10.4	3.7
1900	25°	7.0	9.9	10.8	8.8	2.4
2000	24°	6.6	8.2	8.8	7.2	1.5
2100	24°	6.4	7.2	7.4	5.7	0.6
2200	24°	6.2	5.7	5.6	4.4	0.15
2300	24°	5.8	4.3	4.1	2.9	0.1
2400	*					
0100	*					
0200	23.5°	5.0	1.2	0.7	0.75	0.1
0300	23°	5.2	0.65	0.25	0.4	0.05
0400	23°	5.5	0.5	0.05	0.3	0.05
0500	23°	5.5	0.15	0.15	0.15	0.05
0600	23°	5.6	0.1	0.1	0.15	0.05
0700	23°	5.4	0.2	0.25	0.2	0.1
0800	23°	5.1	0.2	0.15	0.3	0.1
0900	23°	5.2	0.25	0.4	1.0	0.1
1000	23.5°	5.6	0.6	1.1	2.10	0.1

dome 5 - control (measured concentration =0.16 ppb)
dome 7 - 100 mlMeOH(measured concentration = 0.11 ppb)
dome 8 - 10 ppb atrazine (measured concentration = 2.54 ppb)
dome 10 - 100 ppb atrazine (measured concentration = 65.15 ppb)

*no readings taken

TABLE C5.3. DOME STUDY, 25-26 JUNE 1980, GUINEA MARSH STATION, SET IN ZOSTERA MARINA COMMUNITY
(dissolved oxygen concentrations in parts per million)

Time	Ambient Temp.	DO	Dome 5	Dome 7	Dome 6	Dome 8	Dome 10	Dome 9
0900	23°C	5.5	5.4	5.4	4.9	5.2	5.4	5.3
1000	23°	6.1	6.2	6.0	5.6	5.8	5.8	5.6
1100	23.5°	6.5	6.7	6.5	6.0	6.3	5.5	5.0
1200	24°	7.1	7.6	7.3	6.7	7.0	5.8	4.8
1300	24°	7.6	8.7	8.1	7.8	8.1	6.1	4.6
1400	25°	8.2	9.5	9.2	8.7	9.0	6.3	4.6
1500	25°	8.2	9.9	9.5	9.0	9.6	6.1	4.1
1600	25°	7.4	9.6	10.0	9.0	9.3	5.6	3.7
1700	24.5°	6.9	8.8	9.0	8.2	8.4	4.6	2.8
1800	24°	6.6	8.0	8.6	7.4	7.6	3.3	2.0
1900	24°	6.5	6.7	7.4	6.0	6.3	2.2	1.5
2000	23.5°	6.1	5.2	6.1	4.4	4.9	1.2	1.0
2100	23.5°	5.7	3.8	5.0	2.9	3.5	1.05	0.35
2200	23°	5.3	2.5	3.6	2.65	2.4	0.25	0.35
2300	*							
2400	23°	4.8	0.7	2.55	0.35	0.85	0.15	0.20
0100	23°	5.2	0.2	0.75	0.2	0.25	0.1	0.15
0200	23°	4.3	0.2	0.55	0.2	0.15	0.1	0.2
0300	23°	4.7	0.2	0.55	0.1	0.05	0.05	0.2
0400	23°	4.5	0.2	0.45	0.1	0.1	0.05	0.2
0500	*							
0600	23°	4.5	0.35	0.15	0.1	0.15	0.8	0.2
0700	23°	4.8	0.1	0.1	0.1	0.05	0.05	0.1
0800	23°	5.0	0.10	0.75	0.25	2.2	1.3	0.15
0900	23°	5.1	0.3	0.35		0.1	0.05	0.35

dome 5 - control (measured concentration = 0.28ppb)
dome 7 - MeOH (measured concentration = 0.10 ppb)
dome 6 - 1 ppb atrazine (measured concentration = 1.48 ppb)
dome 8 - 10 ppb atrazine (measured concentration = 6.14 ppb)
dome 10- 100 ppb atrazine (measured concentration = 72.49 ppb)
dome 9 - 1000 ppb atrazine (measured concentration = 515.15 ppb)

*no readings taken

TABLE C5.4. DOME STUDY, 15-16 JULY 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY

(dissolved oxygen concentrations in parts per million)

	Ambient							
Time	Temp.	DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0800	25°C	5.0	5.9	5.3	5.5	4.7	4.7	6.1
0900	25°	6.1	8.1	6.8	7.2	5.5	6.0	6.5
1000	25.5°	6.9	6.8	6.8	7.4	7.1	6.3	4.3
1100	26°	7.2	9.5	8.5	9.3	8.6	7.0	5.9
1200	26°	7.3	9.9	9.5	10.4	10.4	7.7	2.6
1300	26.5°	7.9	12.0	10.7	11.2	11.8	8.6	2.4
1400	27°	8.6	13.4	12.3	12.6	13.1	9.2	1.9
1500	27.5°	8.4	14.5	13.4	13.7	14.4	9.4	1.7
1600	28°	8.7	15.4	14.4	14.6	15.2	9.8	1.45
1700	28°	8.6	16.0	15.2	15.2	16.0	9.8	1.1
1800	28°	8.7	15.9	15.4	15.4	15.8	9.5	1.2
1900	28°	8.7	15.8	15.2	15.2	15.4	8.7	0.40
2000	28°	8.0	14.2	13.8	14.0	14.0	7.3	0.20
2100	27.5°	6.8	12.2	12.4	12.6	12.2	5.5	0.10
2200	27°	6.0	10.3	10.8	10.9	10.3	3.8	0.10
2300	27°	5.8	8.3	9.1	8.7	8.4	2.3	0.10
2400	26.5°	6.1	6.5	7.5	7.0	6.6	1.05	0.10
0100	26°	6.2	4.7	5.6	5.2	4.6	0.4	0.10
0200	26°	6.0	3.0	4.05	3.5	2.85	0.2	0.10
0300	26°	5.9	1.7	2.75	2.2	1.5	0.10	0.05
0400	26°	5.6	0.95	1.70	1.25	0.35	0.10	0.05
0500	26°	5.0	0.45	0.9	0.5	0.10	0.05	0.05
0600	25.5°	5.1	0.15	0.35	0.10	0.05	0.05	0.05
0700	25.5°	4.3	0.20	0.15	0.05	0.05	0.05	0.05
0800	25°	4.7	0.25	0.15	0.10	0.05	0.05	0.02
0900	25.5°	5.6	0.95	0.85	0.65	0.35	0.05	0.05
1000	26°	6.9	2.2	1.85	1.55	0.95	0.10	0.05
1100	26.5°	7.1	3.3	2.7	2.3	1.4	0.10	0.05
1200	27°	7.4	4.4	3.7	3.15	2.0	0.10	0.05
1300	27.5°	8.0	5.7	4.75	4.15	2.6	0.15	0.10
1400	28°	9.2	7.3	6.00	5.3	3.45	0.15	0.10
1500	28.5°	9.8	8.7	7.0	6.2	4.2	0.15	0.10

dome 5 - control (measured concentration =0.16 ppb)
dome 6 - MeOH (measured concentration = 0.17 ppb)
dome 7 - 1 ppb atrazine (measured concentration = 1.47 ppb)
dome 8 - 10 ppb atrazine (measured concentration = 6.38 ppb)
dome 9 - 100 ppb atrazine (measured concentration = 71.85 ppb)
dome 10 - 1000 ppb atrazine (measured concentration = 761.90 ppb)

TABLE C5.5. DOME STUDY, 14 JULY 1980, GUINEA MARSH STATION, SET IN ZOSTERA MARINA COMMUNITY (dissolved oxygen concentrations in parts per million)

Time	Temp.	Ambient DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0830	26.5°C	4.2	4.5	4.8	4.8	4.9	4.9	5.0
0930	27°	5.5	5.2	5.3	5.7	6.1	5.6	4.4
1030	27°	6.9	6.4	6.7	7.0	7.9	6.5	3.4
1130	28°	7.1	7.7	8.0	8.4	9.7	7.1	2.5
1230	28°	7.4	8.9	8.9	9.6	11.9	7.5	1.85
1330	29°	7.8	10.1	9.3	10.8	12.4	7.9	1.35
1430	29°	8.4	11.3	9.5	12.0	13.5	8.5	0.9
1530	29.5°	8.6	11.9	9.8	12.6	14.0	9.3	0.65
1630	30°	8.6	12.4	9.2	13.0	13.9	8.3	0.35
1730	30°	8.6	12.8	8.7	13.0	13.4	7.8	0.2
1830	30.5°	8.5	12.2	7.6	12.2	12.2	6.8	0.2

Dome 5 - control (measured concentration 0.16 ppb)
Dome 6 - MeOH (measured concentration 0.17 ppb)
Dome 7 - 1 ppb atrazine (measured concentration 1.47 ppb)
Dome 8 - 10 ppb atrazine (measured concentration 6.38 ppb)
Dome 9 - 100 ppb atrazine (measured concentration 71.85 ppb)
Dome 10- 1000 ppb atrazine (measured concentration 761.90 ppb)

TABLE C5.6. DOME STUDY, 18-19 JULY 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

Time	Ambient Temp.	Ambient DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0800	25.5°C	3.2	3.2	3.3	3.4	3.4	3.3	3.5
0900	25.1°	4.1	3.5	3.6	3.9	3.5	3.8	2.9
1000	25.2°	5.0	4.2	4.7	4.8	4.2	4.5	2.2
1100	26.0°	6.1	5.3	6.0	5.8	5.0	5.2	1.6
1200	26.8°	6.9	5.9	6.7	6.4	5.2	5.4	1.1
1300	26.9°	7.1	6.9	7.3	7.3	5.8	5.7	0.7
1400	27.4°	7.5	8.2	9.2	8.6	6.7	6.4	0.4
1500	27.8°	8.2	9.7	10.4	9.8	7.4	6.9	0.3
1600	28.2°	9.3	10.6	11.4	10.5	7.9	7.1	0.3
1700	28.2°	10.6	11.2	12.0	11.1	7.9	6.9	0.2
1800	28.4°	10.8	11.0	12.0	11.0	7.5	6.5	0.2
1900	28.5°	11.0	10.2	11.3	9.9	6.2	5.4	0.2
2000	28.5°	10.4	9.2	10.2	8.3	4.4	4.3	0.20
2100	28.5°	9.9	7.3	8.3	6.4	2.45	2.95	0.15
2200	28.0°	8.5	5.6	6.5	4.0	0.65	1.60	0.10
2300	28.0°	7.4	3.25	4.05	1.70	0.10	0.50	0.05
2400	28.0°	7.8	2.30	2.70	0.50	0.10	0.10	0.05
0100	28.0°	7.0	2.00	1.10	0.35	0.05	0.05	0.05
0200	28.0°	6.5	0.70	0.35	0.10	0.05	0.05	0.05
0300	27.5°	6.0	0.30	0.15	0.10	0.05	0.05	0.05
0400	28.0°	5.6	0.25	0.20	0.10	0.05	0.05	0.05
0500	28.0°	5.1	0.20	0.10	0.10	0.05	0.05	0.05
0600	27.5°	4.3	0.15	0.10	0.10	0.05	0.05	0.05
0700	27.5°	3.6	0.15	0.15	0.10	0.10	0.05	0.05

Dome 5 - control (measured concentration = 0.21 ppb)
Dome 6 - MeOH (measured concentration = ≤0.10 ppb)
Dome 7 - 1 ppb atrazine (measured concentration = 0.81 ppb)
Dome 8 - 10 ppb atrazine (measured concentration = 4.85 ppb)
Dome 9 - 100 ppb atrazine (measured concentration = 61.44 ppb)
Dome 10- 1000 ppb atrazine (measured concentration = 709.29 ppb)

TABLE C5.7. DOME STUDY, 29 JULY 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

Time	Temp.	Ambient DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0800	27.2	3.06	2.08	1.71	2.24	1.17	1.96	1.99
0900	26.9	5.03	1.70	0.91	1.55	1.03	1.26	1.12
1000	26.9	5.10	1.55	0.57	1.38	0.65	1.08	0.73
1100	27.4	4.70	2.10	0.62	1.90	0.72	1.69	0.73
1200	27.7	5.35	3.25	1.02	3.14	1.12	2.95	1.04
1300	28.0	6.24	3.75	0.95	3.59	1.02	3.30	0.93
1400	28.5	7.00	4.93	1.22	4.73	1.34	4.33	1.56
1500	28.8	7.05	5.56	1.22	5.33	1.33	4.80	1.13
1600	29.2	7.50	6.48	1.26	6.22	1.33	5.58	1.18
1700	29.5	7.95	7.00	1.02	6.65	0.98	5.64	0.83
1800	29.6	8.10	7.06	0.67	6.39	0.44	5.08	0.43
1900	29.4	8.14	6.39	0.16	5.50	0.04	3.83	0.03
2000	29.1	8.62	4.86	0.055	3.55	0.042	1.83	0.03
2100	28.7	7.76	3.28	0.05	1.87	0.04	0.67	0.06
2200	28.3	7.40	2.30	0.13	1.01	0.105	0.20	0.09
2300	28.1	6.62	1.01	0.20	0.20	0.140	0.14	0.07

Dome 5 - control (measured concentration = 0.24 ppb)
Dome 6 - 51% Shade (measured concentration = 0.24 ppb)
Dome 7 - MeOH (measured concentration = 0.58 ppb)
Dome 8 - MeOH 51% (measured concentration = 0.58 ppb)
Dome 9 - 10 ppb atrazine (measured concentration = 6.51 ppb)
Dome 10-10 ppb 51% (measured concentration = 9.87 ppb)

TABLE C5.8. DOME STUDY, 30 JULY 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

	Ambient							
Time	Temp.	DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0830	27.0	3.38	3.14	2.82	3.33	2.69	3.10	2.74
0930	27.1	4.70	3.23	2.13	3.77	1.89	3.09	2.29
1030	27.6	6.20	4.06	1.83	4.60	1.48	3.47	1.90
1130	27.6	6.72	4.92	1.77	5.54	1.30	4.05	1.81
1230	28.0	6.80	5.96	1.94	6.55	1.42	4.65	1.86
1330	28.4	7.75	7.07	2.22	7.57	1.61	5.42	2.09
1430	28.7	8.16	8.40	2.48	8.65	1.73	6.22	2.85
1530	29.0	8.55	8.27	2.38	8.49	1.70	6.58	2.40
1630	29.2	8.60	9.18	2.56	9.08	1.88	6.95	2.34
1730	29.4	8.50	9.65	2.42	9.46	1.55	7.26	2.07
1830	29.3	8.68	9.07	1.58	8.69	1.09	6.45	1.50
1930	29.1	7.82	8.20	1.16	7.29	0.90	4.49	0.85
2030	28.8	6.86	6.23	0.45	5.07	0.30	2.75	0.28

Dome 5 - control (measured concentration = 0.18 ppb)
Dome 6 - control 51%
Dome 7 - MeOH
Dome 8 - MeOH -51%
Dome 9 - 10 ppb atrazine (measured concentration = 6.30 ppb)
Dome 10- 10 ppb - 51% (measured concentration = 7.86 ppb)

TABLE C5.9. DOME STUDY, 31 JULY 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

	Ambient							
Time	Temp.	DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0830	27.6	2.88	2.71	2.94	2.94	2.56	2.54	1.42
0930	27.5	5.37	2.93	4.34	3.09	2.20	2.55	0.71
1030	27.6	6.82	3.79	6.83	4.00	1.21	3.02	0.58
1130	28.0	7.60	4.67	8.24	5.38	1.15	3.67	0.46
1230	28.2	8.00	5.51	8.85	8.20	1.22	4.16	0.47
1330	28.6	8.73	6.17	9.05	5.69	1.02	4.39	0.34
1430	29.0	9.34	6.74	9.49	6.12	0.96	4.62	0.22
1530	29.3	9.32	6.98	9.78	5.88	1.98	5.95	1.18
1630	29.7	9.75	7.80	11.95	-	-	4.42	1.70
1700	-	10.2	6.6	9.4	4.9	0.7	3.1	0.15
1800	30.2	10.0	6.3	*	4.0	0.35	2.4	0.15
1900	30.1	9.7	5.0	*	1.7	0.25	4.20	0.20
2000	29.8	8.7	3.1	*	0.2	0.2	0.15	0.10
2100	29.6	8.3	1.65	*	0.15	0.15	0.10	0.05

Dome 5 - control
Dome 6 - control 51% shade
Dome 7 - MeOH
Dome 8 - MeOH 51% shade
Dome 9 - 10 ppb atrazine (measured concentration 6.51 ppb)
Dome 10 - 10 ppb - 51% shade (measured concentration 8.81 ppb)

* discontinued due to pump failure

TABLE C5.10. DOME STUDY, 1 AUGUST 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

Time	Ambient Temp.	Ambient DO	Dome 5	Dome 6	Dome 7	Dome 10
0900	28.0	4.02	4.37	4.03	4.46	4.10
1000	27.9	6.38	4.67	3.47	4.81	3.14
1100	28.2	6.73	5.05	3.28	5.41	2.97
1200	28.6	7.08	5.71	3.38	5.80	2.80
1300	29.0	7.15	6.17	3.28	5.98	2.41
1400	29.3	7.80	6.66	3.15	6.03	1.91
1500	29.8	7.64	7.03	3.09	6.00	1.59
1600	29.8	7.40	7.37	2.83	5.73	1.10

Dome 5 - control
Dome 6 - control 30%
Dome 7 - 10 ppb
Dome 10-10 ppb 30%

No water samples taken.

TABLE C5.11. DOME STUDY, 12 AUGUST 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY (dissolved oxygen concentrations in parts per million)

Time	Temp.	Ambient DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0930	28.9	5.90	5.39	5.72	5.71	5.67	5.69	5.41
1030	28.5	6.59	4.03	4.44	4.71	4.10	4.65	3.38
1130	28.8	6.97	3.53	3.83	4.40	3.29	4.32	2.38
1230	29.1	7.05	3.54	3.46	4.39	2.94	4.39	1.85
1330	29.6	7.34	3.77	3.24	4.73	2.85	4.69	1.63
1430	29.7	7.39	3.95	3.00	5.00	2.66	4.82	1.39
1530	30.8	8.00	4.25	2.91	5.29	2.68	5.09	1.47
1630	30.1	8.94	4.98	3.38	6.18	3.14	5.93	1.92
1730	30.4	9.56	5.07	3.40	6.56	3.09	6.09	1.96
1830	30.5	8.75	4.61	2.82	6.27	2.55	5.66	1.58
1930	30.5	8.58	3.32	1.86	5.20	1.59	4.43	0.84
2030	30.3	8.24	1.70	1.06	3.54	0.97	2.89	0.38

Dome 5 - control
Dome 6 - control 30%
Dome 7 - 1 ppb atrazine (measured concentration = 0.63 ppb)
Dome 8 - 1ppb 30% (measured concentration = 0.36 ppb)
Dome 9 - 10 ppb (measured concentration = 7.41 ppb)
Dome 10-10 ppb 30% (measured concentration = 6.93 ppb)

TABLE C5.12. DOME STUDY, 13 AUGUST 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

	Ambient							
Time	Temp.	DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0830	26.1	3.14	2.89	3.12	3.09	3.16	3.18	3.19
0930	26.7	3.90	3.46	2.50	2.60	2.64	3.63	3.22
1030	28	9.0	5.60	2.80	5.60	3.00	5.80	4.30
1130	28	11.0	6.20	3.00	6.20	3.00	6.30	4.45
1230	28	9.20	7.70	3.35	7.20	3.70	7.60	4.85
1330	28	12.6	8.80	4.50	9.80	4.90	9.80	5.80
1430	28	11.4	10.2	5.10	10.40	5.40	10.80	6.30
1530	28.5	13.6	11.25	5.7	12.9	6.0	12.65	6.90
1630	29	14.5	12.40	5.9	13.6	6.0	13.20	6.80
1730	29	18.9	13.0	5.3	13.8	5.5	13.4	6.50
1830	29	18.4	11.4	3.55	12.4	3.75	12.0	5.10
1930	29	15.5	9.1	2.00	10.6	2.00	10.1	3.25
2030	28.5	16.4	5.1	0.75	7.5	0.75	7.00	1.35

Dome 5 - control
Dome 6 - control 30%
Dome 7 - 1 ppb atrazine (measured concentration = 0.72 ppb)
Dome 8 - 1 ppb 30% (measured concentration = 0.69 ppb)
Dome 9 - 10 ppb atrazine (measured concentration = 6.58 ppb)
Dome 10-10 ppb 30% (measured concentration = 6.35 ppb)

TABLE C5.13. DOME STUDY, 14 AUGUST 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

Time	Temp.	Ambient DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0830	27	4.20	3.40	3.20	3.65	3.40	3.50	3.30
0930	27	6.20	3.40	2.50	3.50	2.70	3.00	2.40
1030	27	8.20	3.30	1.90	3.40	2.20	2.80	1.80
1130	27.5	8.20	3.60	1.80	3.80	2.20	3.10	1.65
1230	28	8.50	4.35	2.30	4.30	2.90	3.70	2.05
1330	28.5	10.00	5.15	2.80	4.95	3.50	4.25	2.45
1430	29	9.8	6.00	3.00	5.15	3.75	4.45	2.50
1530	29	10.20	5.50	2.55	4.40	3.00	3.35	1.70
1630	29	10.10	5.60	2.20	3.60	2.30	2.50	1.70
1730	29	9.20	4.80	1.90	2.75	1.55	1.40	0.55
1830	29	8.20	3.80	1.05	1.30	-	-	-
1930	29	7.90	2.60	-	-	-	-	0.20
2030	29	7.70	1.40	0.55	0.15	0.15	0.10	0.05
2130	28	10.00	0.25	0.30	0.15	0.10	0.10	0.10
2230	28	9.00	0.25	0.25	0.15	0.10	0.10	0.10
2330	28	8.40	0.20	0.20	0.10	0.10	0.05	0.05

Dome 5 - control
Dome 6 - control 30%
Dome 7 - 1 ppb atrazine (measured concentration = 1.02 ppb)
Dome 8-1 ppb 30% (measured concentration = 0.85 ppb)
Dome 9 - 10 ppb atrazine (measured concentration = 7.18 ppb)
Dome 10- 10 ppb 30% (measured concentration = 6.54 ppb)

TABLE C5.14. DOME STUDY, 15 AUGUST 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

	Ambient							
Time	Temp.	DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0830	26.9	5.60	4.65	4.30	4.65	4.25	4.35	4.25
0930	27	5.40	4.20	2.90	3.40	2.60	2.70	2.70
1030	27.5	7.20	4.30	2.10	2.90	1.60	2.00	1.70
1130	28	7.80	4.70	1.80	2.60	1.00	1.75	1.20
1230	28	8.00	5.40	1.65	2.55	0.95	1.80	1.05
1330	28.5	8.20	5.70	1.55	2.20	-	1.75	0.85
1430	29.0	8.30	5.80	1.35	1.80	0.80	1.35	0.60
1530	29.0	8.30	6.00	1.20	1.35	0.40	1.00	0.35
1630	29	8.00	5.70	0.90	1.05	0.25	0.85	0.30
1730	28.5	7.80	5.50	0.60	0.85	0.10	0.55	0.05

Dome 5 - control
Dome 6 - control 30%
Dome 7 - 1 ppb atrazine
Dome 8 - 1 ppb 30%
Dome 9 - 10 ppb atrazine
Dome 10-10 ppb 30%

No water samples taken.

TABLE C5.15. DOME STUDY, 8 SEPTEMBER 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY (dissolved oxygen concentrations in parts per million)

Time	Temp.	Ambient DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0930	25	3.2	3.4	3.2	3.8	3.4	3.6	3.2
1030	25	3.5	3.5	2.9	3.7	3.2	4.0	3.4
1130	25	4.2	4.3	3.0	4.4	3.0	4.6	4.4
1230	25.5	5.4	5.0	3.3	5.1	3.8	5.5	4.8
1330	26.0	6.8	6.4	3.8	6.6	4.2	6.7	5.8
1430	27.0	8.2	7.6	4.6	7.8	4.7	8.6	6.9
1530	27.5	9.5	8.6	5.2	8.7	5.0	9.4	7.9
1630	27.5	9.7	8.5	4.9	8.6	4.3	9.6	7.7
1730	27.5	9.8	8.5	4.3	8.3	3.7	9.4	7.3
1830	27.5	8.0	7.7	3.6	7.1	2.6	8.4	6.2
1930	26.5	7.5	6.3	2.8	5.7	1.7	7.0	4.8
2030	26	7.3	4.8	1.4	3.9	1.0	5.5	3.0

Dome 5 - control
Dome 6 - control 20% (measured concentration = 0.13 ppb)
Dome 7 - 1 ppb atrazine
Dome 8 - 1 ppb 20% (measured concentration = 0.60 ppb)
Dome 9 - 10 ppb atrazine
Dome 10 - 10 ppb 20% (measured concentration = 6.59 ppb)

TABLE C5.16. DOME STUDY, 9 SEPTEMBER 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY (dissolved oxygen concentrations in parts per million)

	Ambient							
Time	Temp.	DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0830	25	4.2	3.7	3.7	3.8	3.8	3.7	3.8
0930	25.5	4.2	3.5	3.2	3.6	3.4	3.6	3.4
1030	25.5	3.7	3.3	2.8	3.4	3.0	3.3	3.1
1130	26	3.8	3.6	2.9	3.7	3.1	3.6	3.2
1230	26	4.6	4.6	3.4	4.6	3.6	4.4	3.7
1330	26.5	4.5	5.5	3.7	5.5	3.9	5.2	3.9
1430	27	4.6	6.2	4.1	6.4	4.2	5.8	4.3
1530	27	4.1	6.8	4.3	6.9	4.2	5.7	4.3
1630	27	4.1	6.7	3.8	6.5	3.6	4.6	3.8
1730	27	4.6	5.4	2.8	5.3	2.6	3.1	3.0

Dome 5 - control
Dome 6 - control 20%
Dome 7 - 1 ppb atrazine
Dome 8 - 1 ppb 20%
Dome 9 - 10 ppb atrazine
Dome 10- 10 ppb 20%

No water samples taken.

TABLE C5.17. DOME STUDY, 10 SEPTEMBER 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

	Ambient							
Time	Temp.	DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0830	24.5	4.2	4.2	4.3	4.2	4.0	4.1	4.1
0930	25.0	4.3	3.5	3.6	3.6	3.3	3.4	3.3
1030	25.0	4.4	3.3	3.2	3.2	3.0	3.1	2.9
1130	25.0	4.3	2.8	2.9	2.6	2.8	2.6	2.3
1230	24.5	4.0	2.5	2.4	2.2	2.5	2.3	1.8
1330	24	4.0	1.8	2.0	1.5	1.9	1.9	1.3
1430	24	4.2	1.8	1.7	1.3	1.8	1.7	1.0
1530	24	4.3	1.6	1.3	1.0	1.5	1.5	0.8
1630	24	4.3	1.4	1.0	0.7	1.2	1.2	0.5
1730	23.5	4.2	1.2	0.8	0.3	0.8	1.0	0.3
1830	23.5	4.0	0.80	0.40	0.20	0.60	0.70	0.20
1930	23.5	3.9	0.60	0.20	0.20	0.50	0.40	0.20

Dome 5 - control
Dome 6 - control 20% (measured concentration = ≤0.10 ppb)
Dome 7 - 1 ppb atrazine
Dome 8 - 1 ppb 20% (measured concentration = 0.26 ppb)
Dome 9 - 10 ppb atrazine
Dome 10 - 10 ppb 20% (measured concentration = 6.79 ppb)

TABLE C5.18. DOME STUDY, 11 SEPTEMBER 1980, GUINEA MARSH STATION, SET IN *ZOSTERA MARINA* COMMUNITY
(dissolved oxygen concentrations in parts per million)

Time	Temp.	Ambient DO	Dome 5	Dome 6	Dome 7	Dome 8	Dome 9	Dome 10
0830	21.5	3.9	4.0	4.0	4.1	4.0	4.0	4.0
0930	22	4.2	3.9	3.8	3.8	3.6	3.9	3.8
1030	23	4.5	3.9	3.4	3.6	3.2	3.7	3.3
1130	23.5	5.0	4.2	3.4	3.7	3.2	4.2	3.2
1230	23.5	5.5	4.6	3.3	3.9	3.2	4.4	3.3
1330	22.5	5.1	5.0	3.3	4.2	3.3	5.0	3.5
1430	22.8	5.1	5.6	3.1	4.4	3.2	5.4	3.2
1530	23.5	6.0	6.2	3.6	4.9	3.6	6.1	3.8
1630	24.5	6.7	7.3	4.1	5.6	4.0	6.9	4.2
1730	25	7.1	7.6	4.0	5.8	4.0	7.2	4.0
1830	24	6.3	7.6	3.6	4.8	3.4	6.6	3.4

Dome 5 - control
Dome 6 - control 20% (measured concentration = $\leq$0.10 ppb)
Dome 7 - 1 ppb atrazine
Dome 8 - 1 ppb 20% (measured concentration = 0.59 ppb)
Dome 9 - 10 ppb atrazine
Dome 10- 10 ppb 20% (measured concentration = 7.77 ppb)

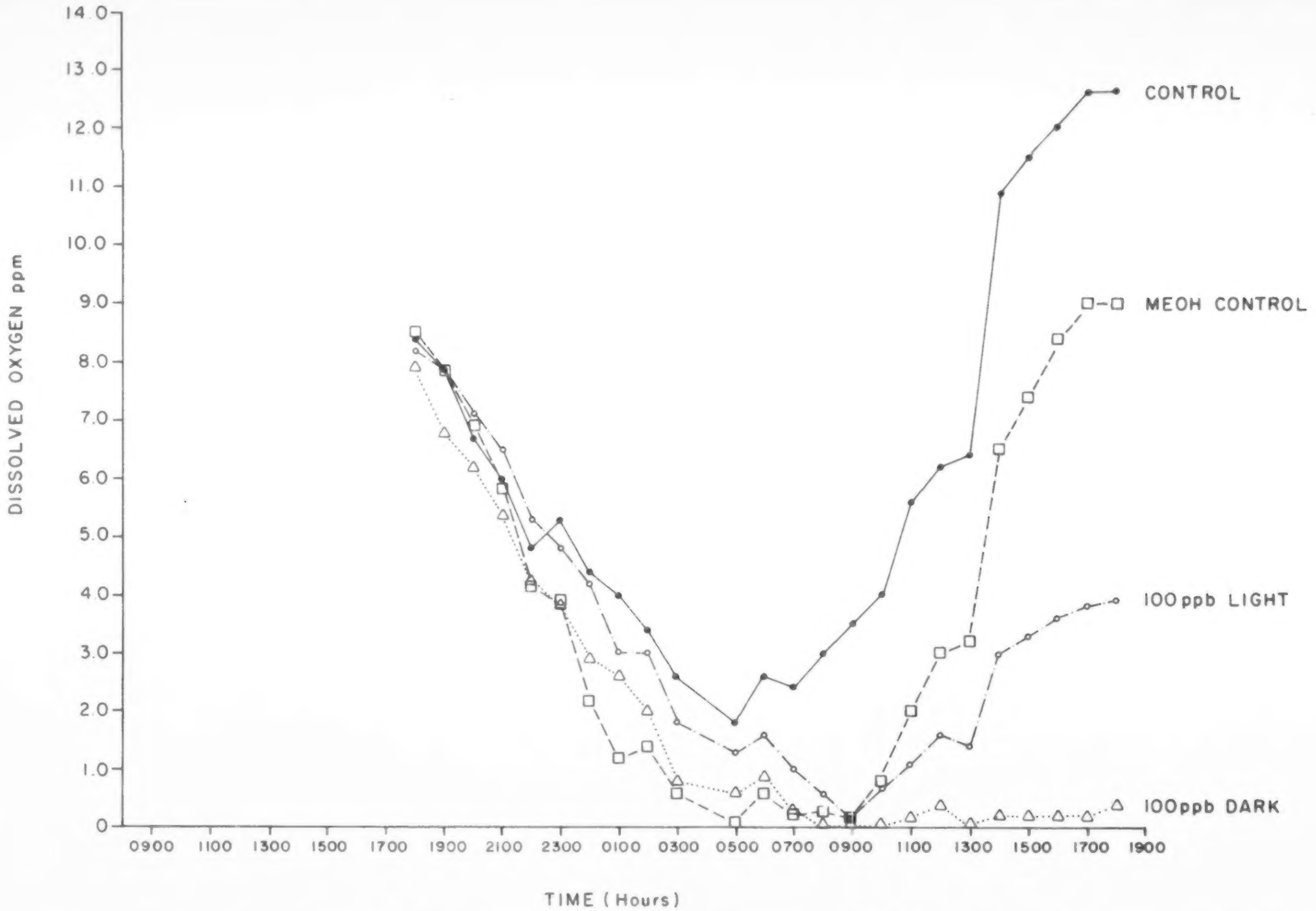

Figure C5.1. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 29-30 May 1980, involving control, methanol control, 100 ppb atrazine "light" and 100 ppb atrazine "dark" domes. Refer to Tables 5.1 (DO vs. Time) and 5.19 (mg O_2 m^{-2} hr^{-1} vs. Time).

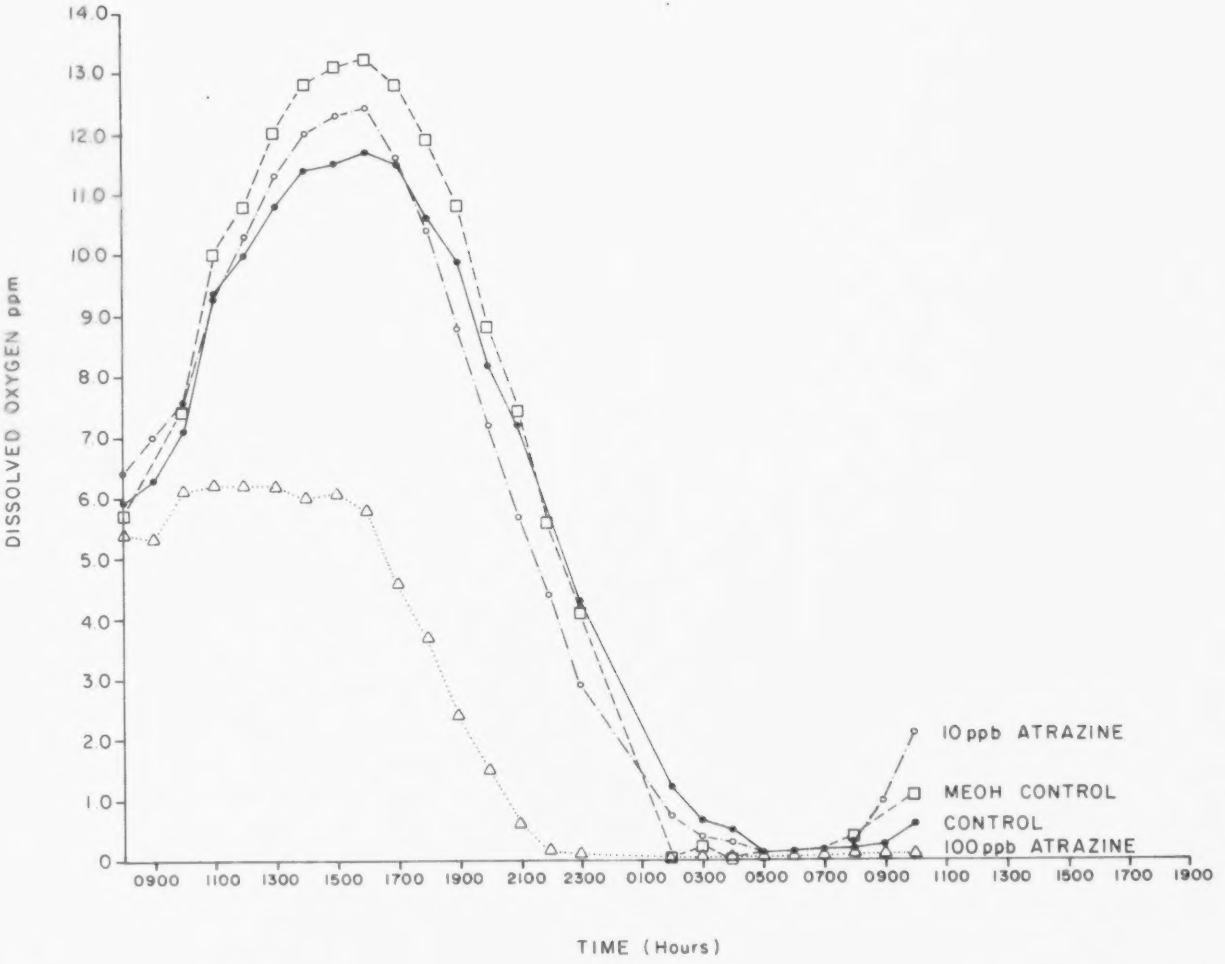

Figure C5.2. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 23-24 June 1980, involving control, methanol control, 10 ppb atrazine and 100 ppb atrazine domes. Refer to Tables 5.2 (DO vs. Time) and 5.20 (mg O_2 m^{-2} hr^{-1} vs. Time).

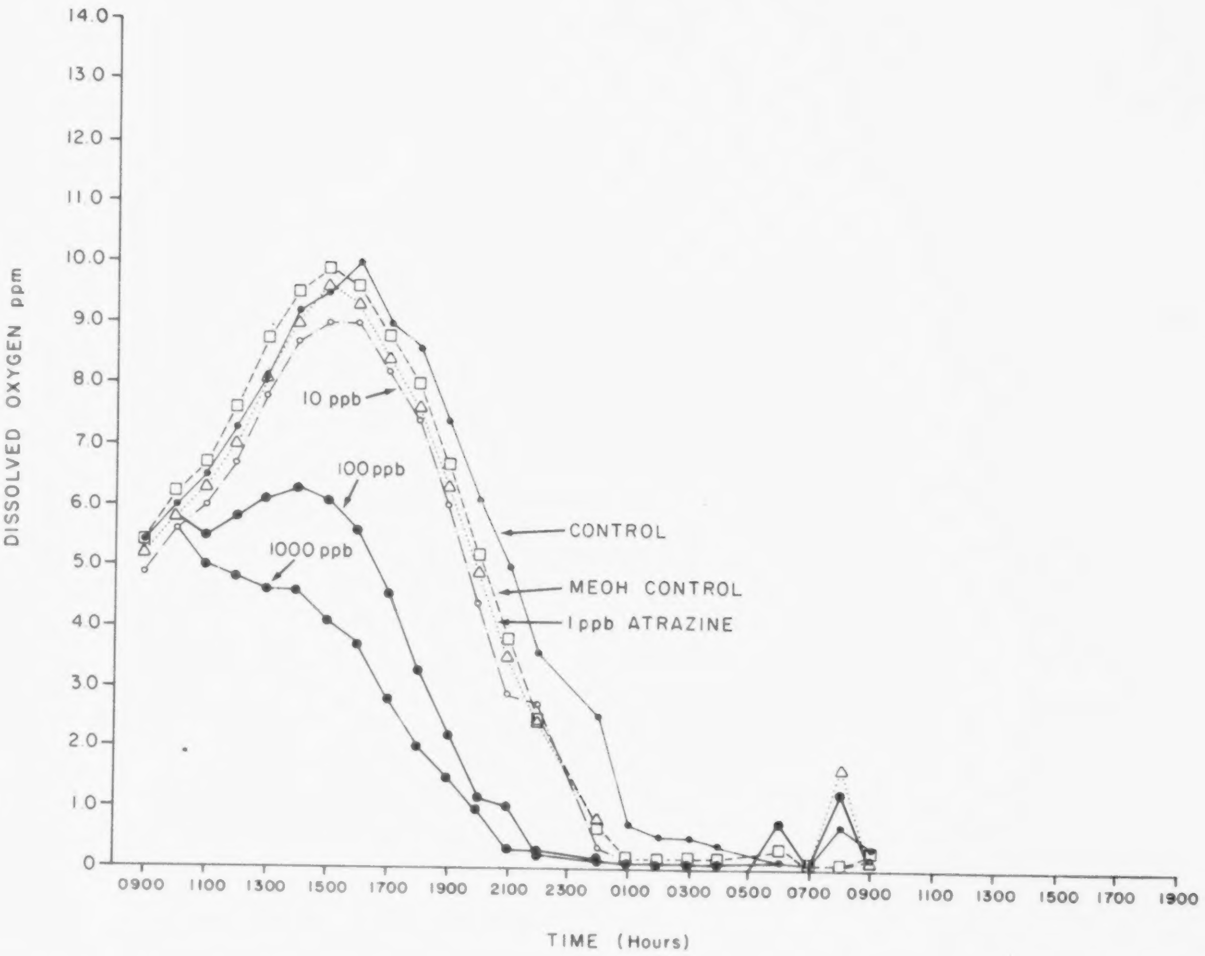

Figure C5.3. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 25-26 June 1980, involving control, methanol control, 1 ppb atrazine, 10 ppb atrazine, 100 ppb atrazine and 1000 ppb atrazine domes. Refer to Tables 5.3 (DO vs. Time) and 5.21 (mg O_2 m^{-2} hr^{-1} vs. Time).

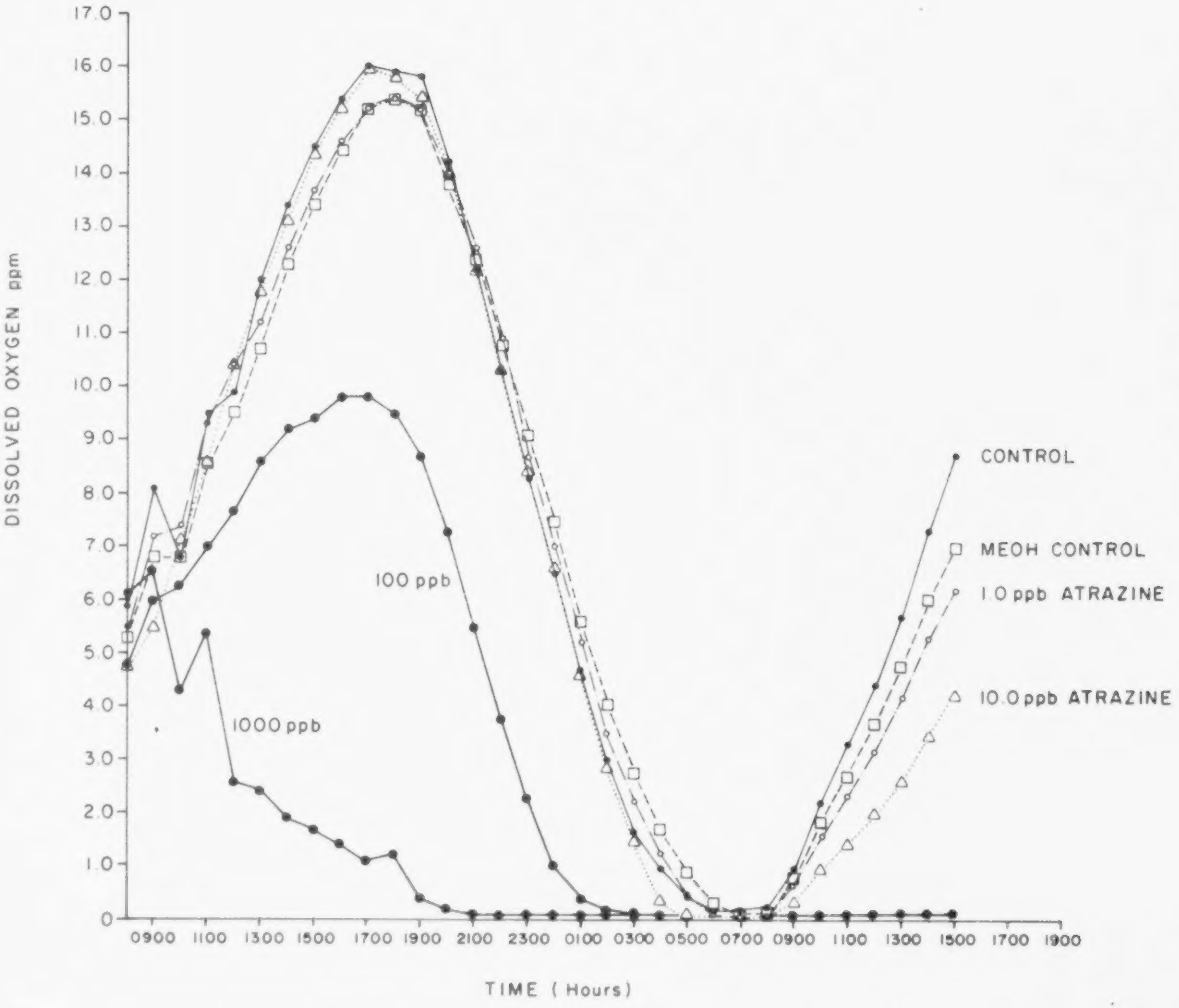

Figure C5.4. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 15-16 July 1980, involving control, methanol control, 1 ppb atrazine, 10 ppb atrazine, 100 ppb atrazine and 1000 ppb atrazine domes. Refer to Tables 5.4 (DO vs. Time) and 5.22 (mg O_2 m^{-2} hr $^{-1}$ vs. Time).

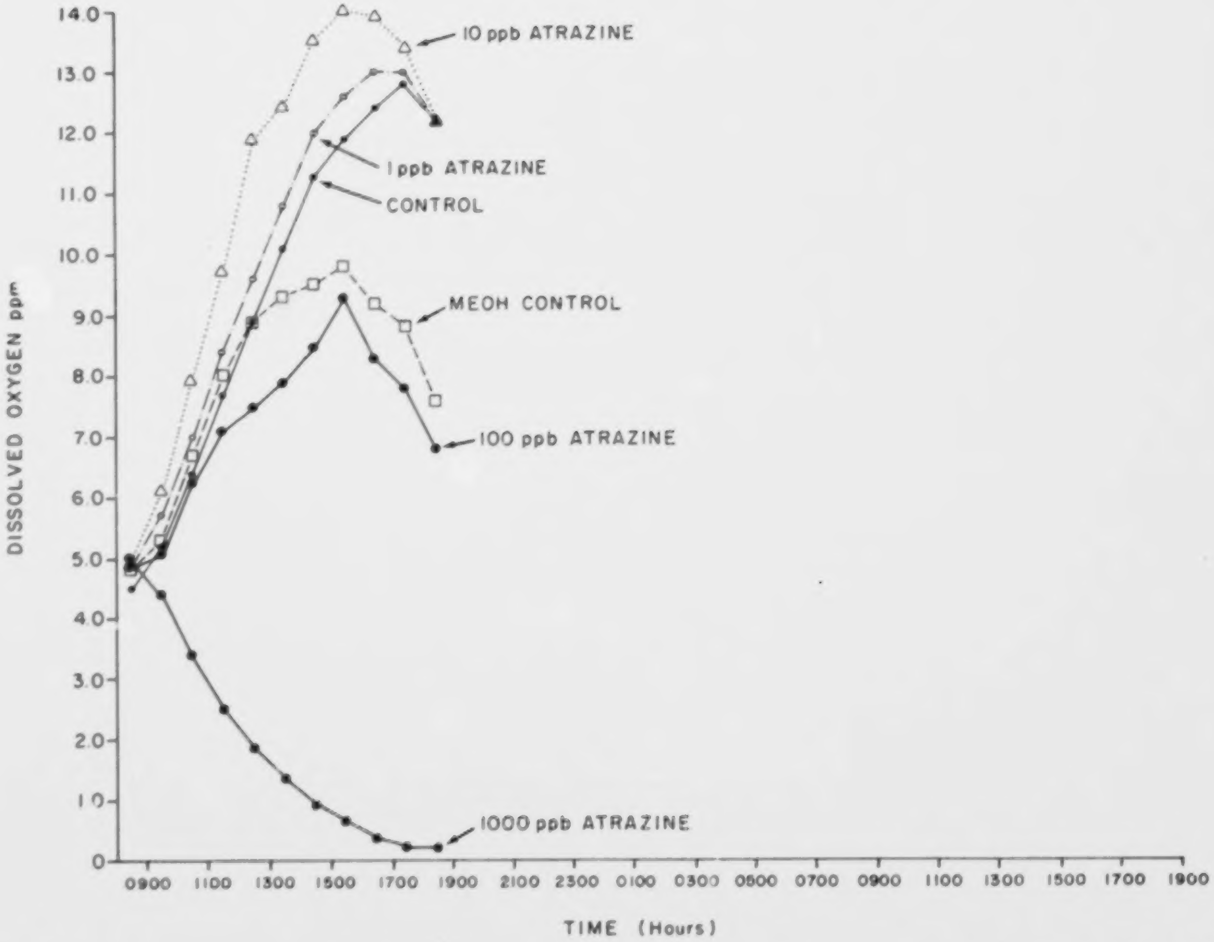

Figure C5.5. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 14 July 1980, involving control, methanol control, 1 ppb atrazine, 10 ppb atrazine, 100 ppb atrazine and 1000 ppb atrazine domes. Refer to Tables 5.5 (DO vs. Time) and 5.23 (mg O_2 m^{-2} hr^{-1} vs. Time).

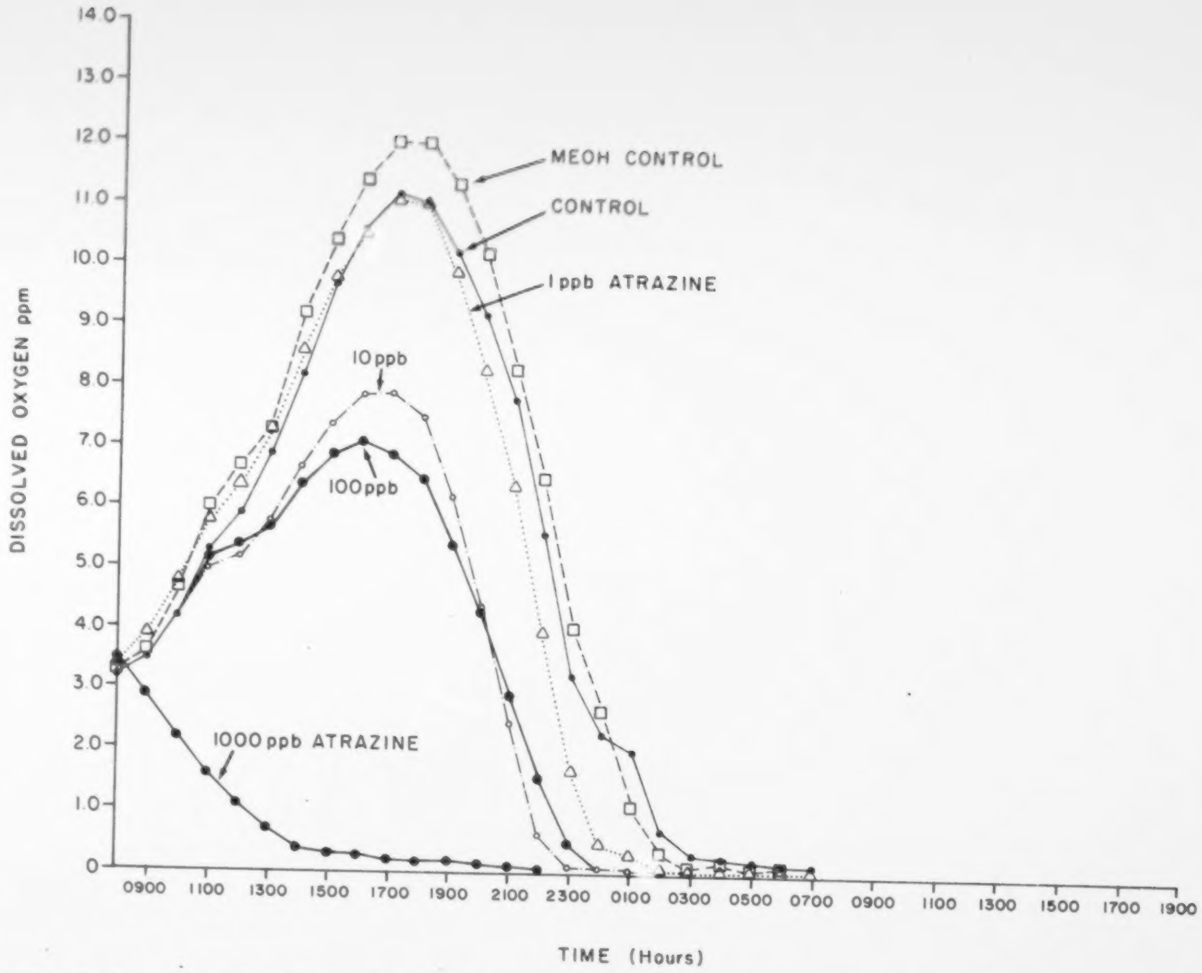

Figure C5.6. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 18-19 July 1980, involving control, methanol control, 1 ppb atrazine, 10 ppb atrazine, 100 ppb atrazine and 1000 ppb atrazine domes. Refer to Tables 5.6 (DO vs. Time) and 5.24 (mg O_2 m^{-2} hr^{-1} vs. Time).

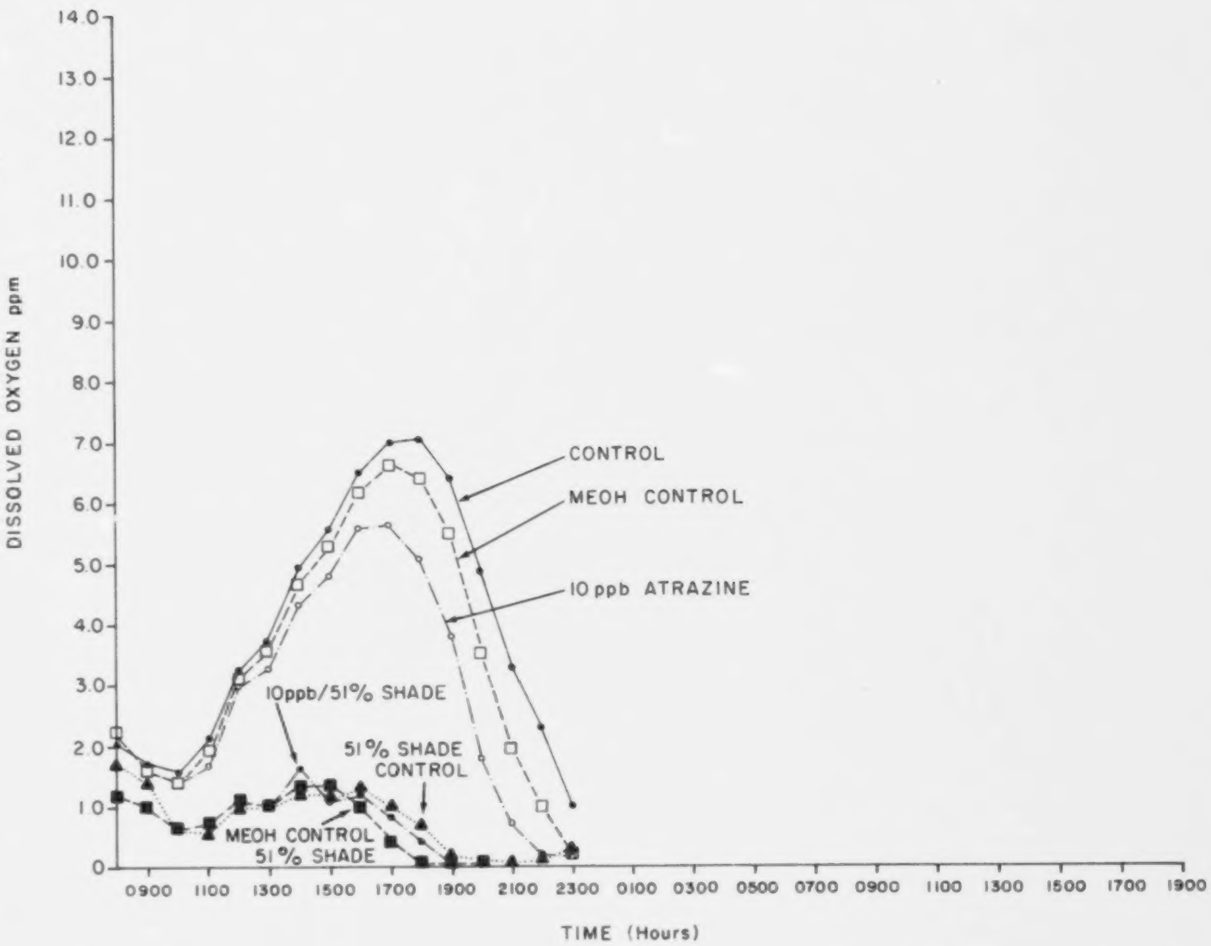

Figure C5.7. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 29 July 1980, involving control, shaded control (51%), methanol control, shaded methanol control (51%), 10 ppb atrazine and shaded 10 ppb atrazine (51%) domes. Refer to Tables 5.7 (DO vs. Time) and 5.25 (mg O_2 m^{-2} hr^{-1} vs. Time).

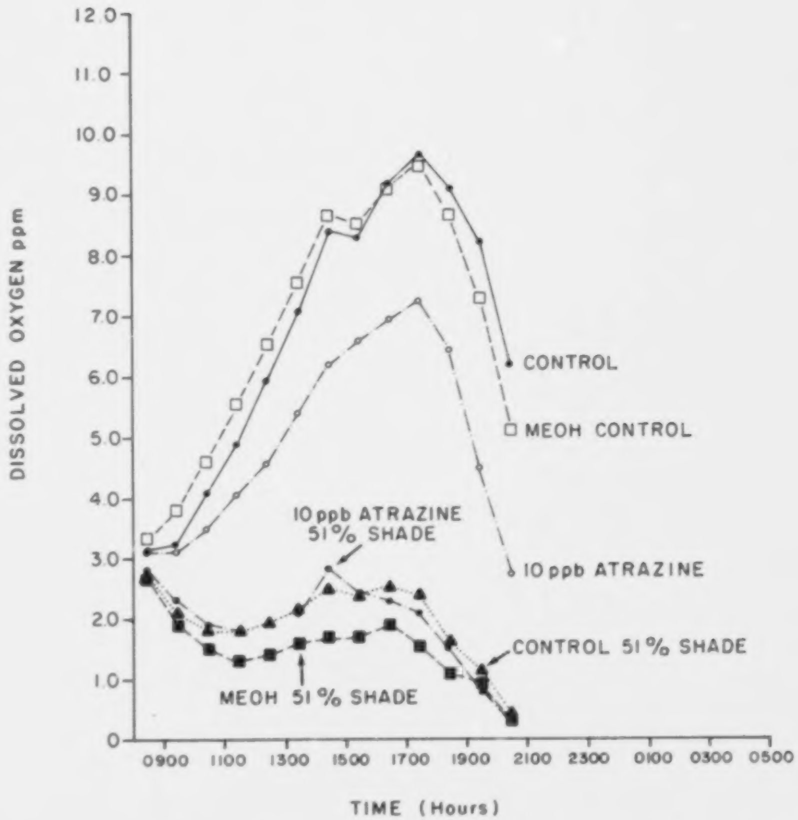

Figure C5.8. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 30 July 1980, involving control, shaded control (51%), methanol control, shaded methanol control (51%), 10 ppb atrazine and shaded 10 ppb atrazine (51%) domes. Refer to Tables 5.8 (DO vs. Time) and 5.26 (mg O_2 m^{-2} hr^{-1} vs. Time).

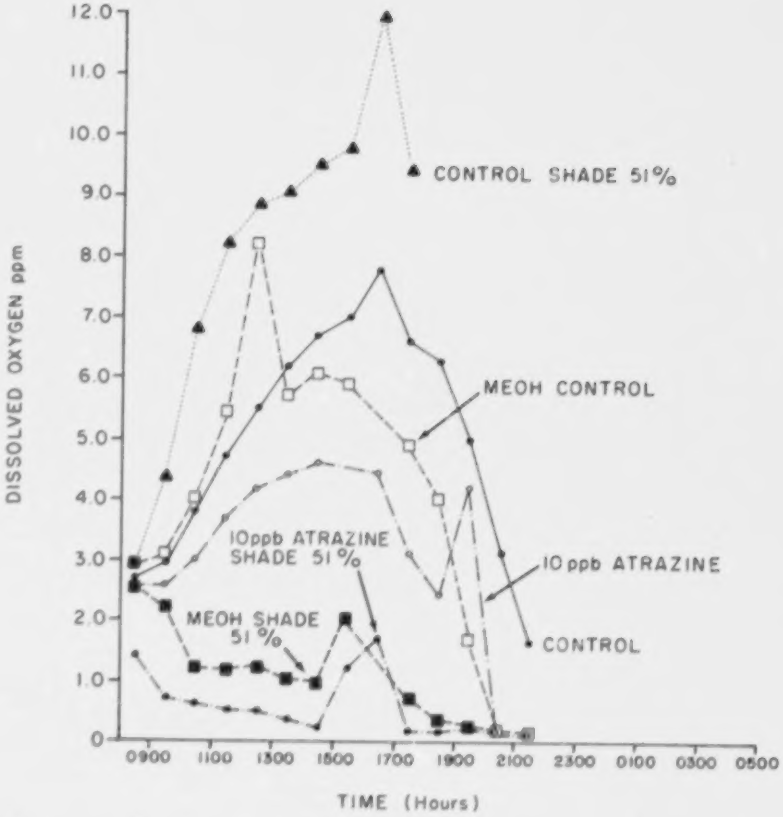

Figure C5.9. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 31 July 1980, involving control, shaded control (51%), methanol control, shaded methanol control (51%), 10 ppb atrazine and shaded 10 ppb atrazine (51%) domes. Refer to Tables 5.9 (DO vs. Time) and 5.27 (mg O_2 m^{-2} hr^{-1} vs. Time).

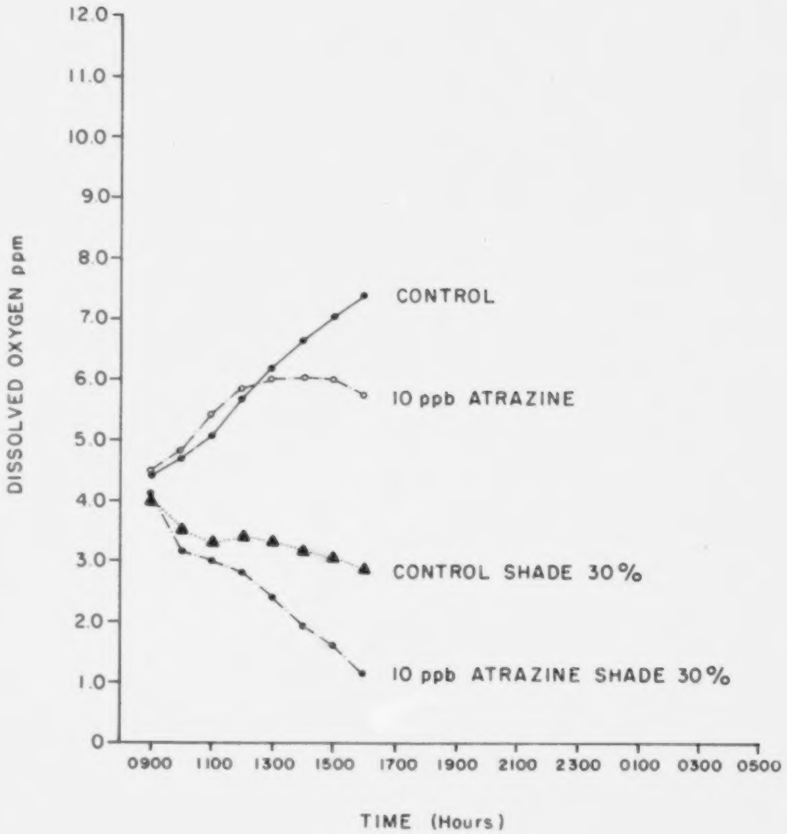

Figure C5.10. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 1 August 1980, involving control, shaded control (30%), 10 ppb atrazine and shaded 10 ppb atrazine (30%) domes. Refer to Tables 5.10 (DO vs. Time) and 5.28 (mg O_2 m^{-2} hr^{-1} vs. Time).

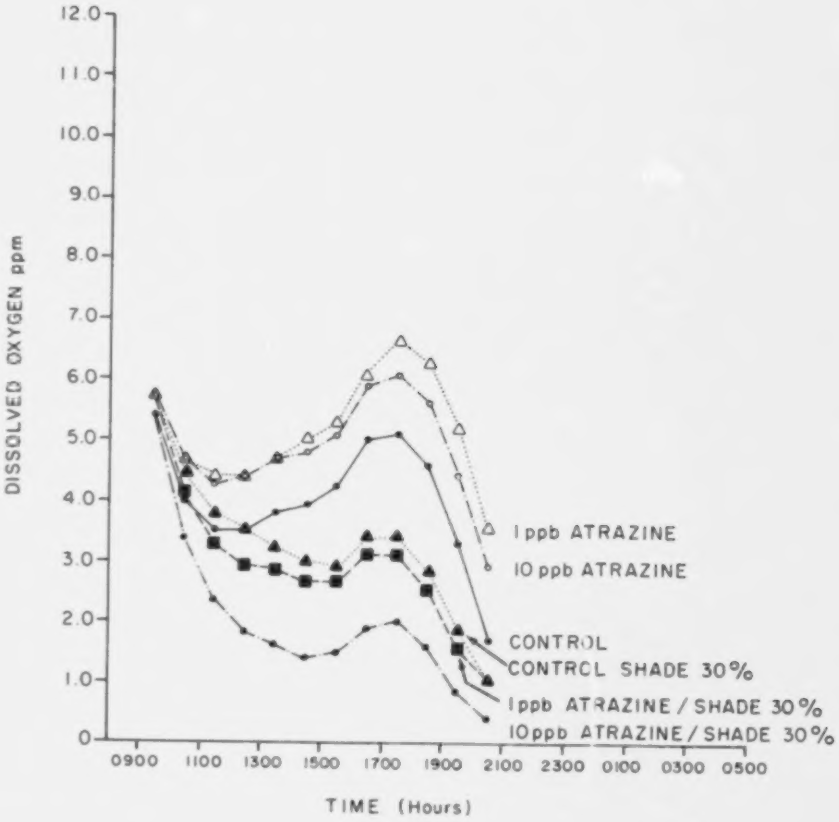

Figure C5.11. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 12 August 1980, involving control, shaded control (30%), 1 ppb atrazine and shaded 1 ppb atrazine (30%), 10 ppb atrazine and shaded 10 ppb atrazine (30%) domes. Refer to Tables 5.11 (DO vs. Time) and 5.29 (mg O_2 m^{-2} hr^{-1} vs. Time).

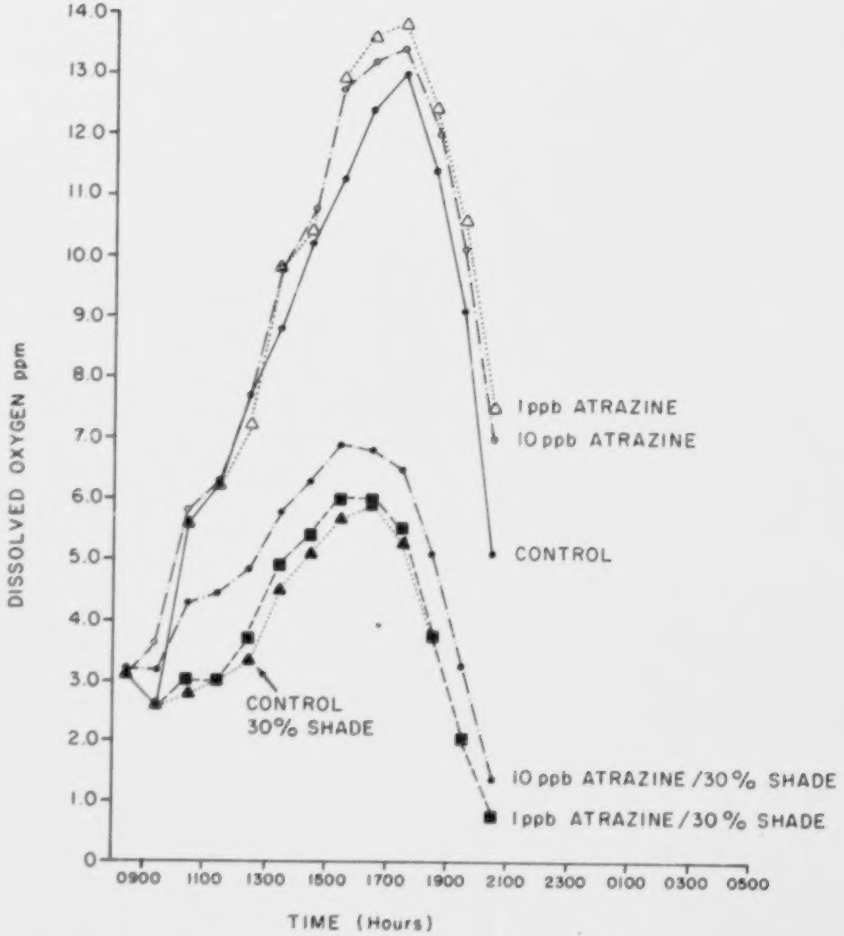

Figure C5.12. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 13 August 1980, involving control, shaded control (30%), 1 ppb atrazine, shaded 1 ppb atrazine (30%), 10 ppb atrazine and shaded 10 ppb atrazine (30%) domes. Refer to Tables 5.12 (DO vs. Time) and 5.30 (mg O_2 m^{-2} hr^{-1} vs. Time).

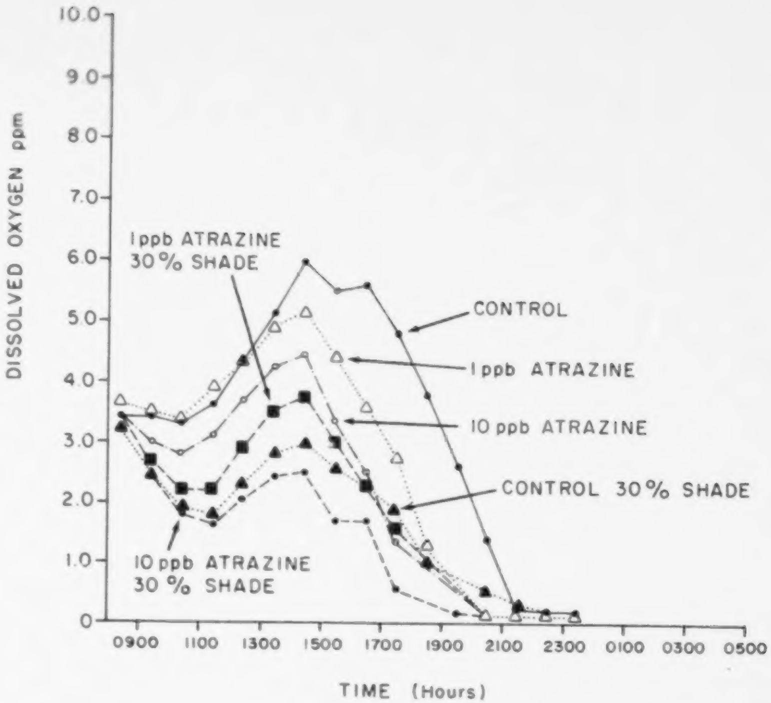

Figure C5.13. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 14 August 1980, involving control, shaded control (30%), 1 ppb atrazine, shaded 1 ppb atrazine (30%), 10 ppb atrazine and shaded 10 ppb atrazine (30%) domes. Refer to Tables 5.13 (DO vs. Time) and 5.31 (mg O_2 m^{-2} hr^{-1} vs. Time).

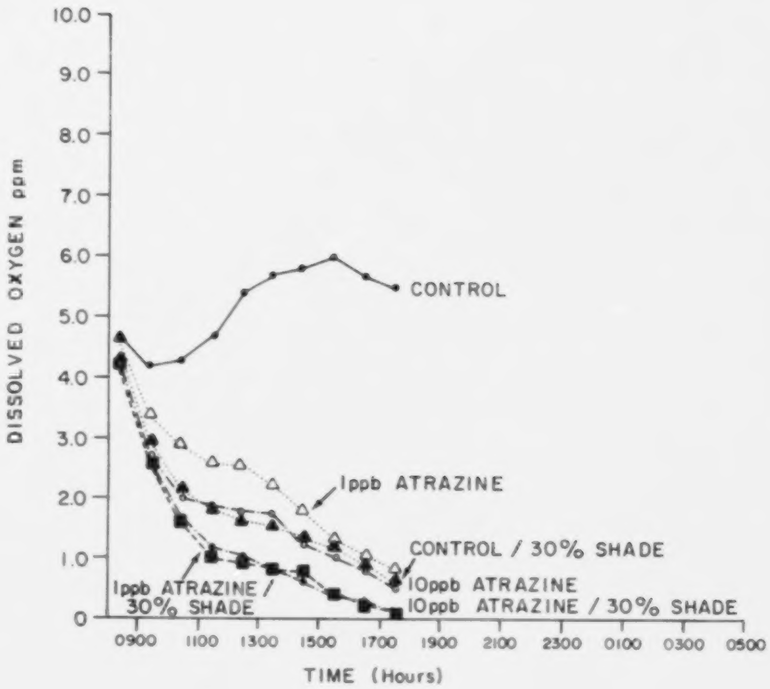

Figure C5.14. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 15 August 1980, involving control, shaded control (30%), 1 ppb atrazine, shaded 1 ppb atrazine (30%), 10 ppb atrazine and shaded 10 ppb atrazine (30%) domes. Refer to Tables 5.14 (DO vs. Time) and 5.32 (mg O_2 m^{-2} hr^{-1} vs. Time).

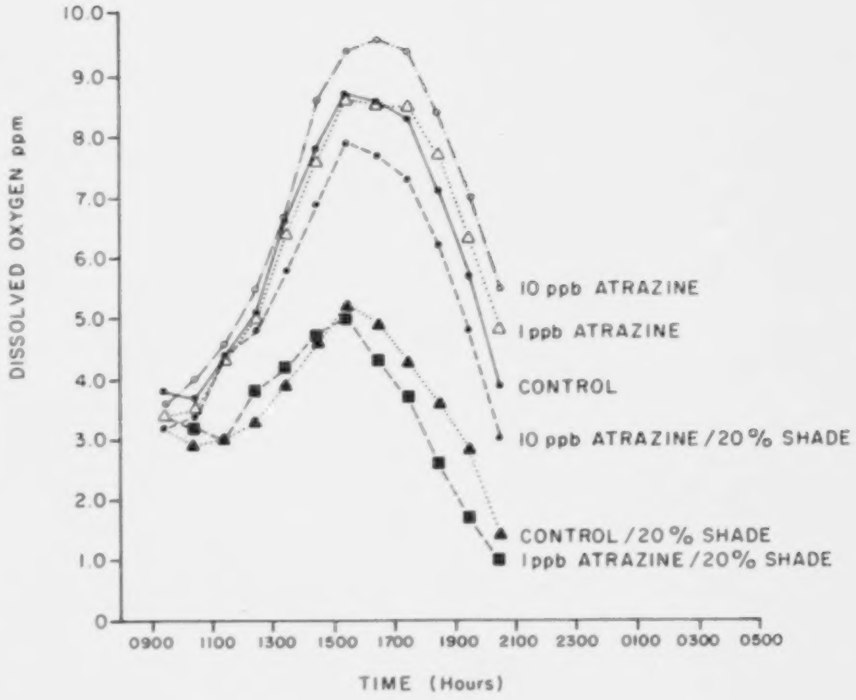

Figure C5.15. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 8 September 1980, involving control, shaded control (20%), 1 ppb atrazine, shaded 1 ppb atrazine (20%), 10 ppb atrazine and shaded 10 ppb atrazine (20%) domes. Refer to Tables 5.15 (DO vs. Time) and 5.33 (mg O_2 m^{-2} hr^{-1} vs. Time).

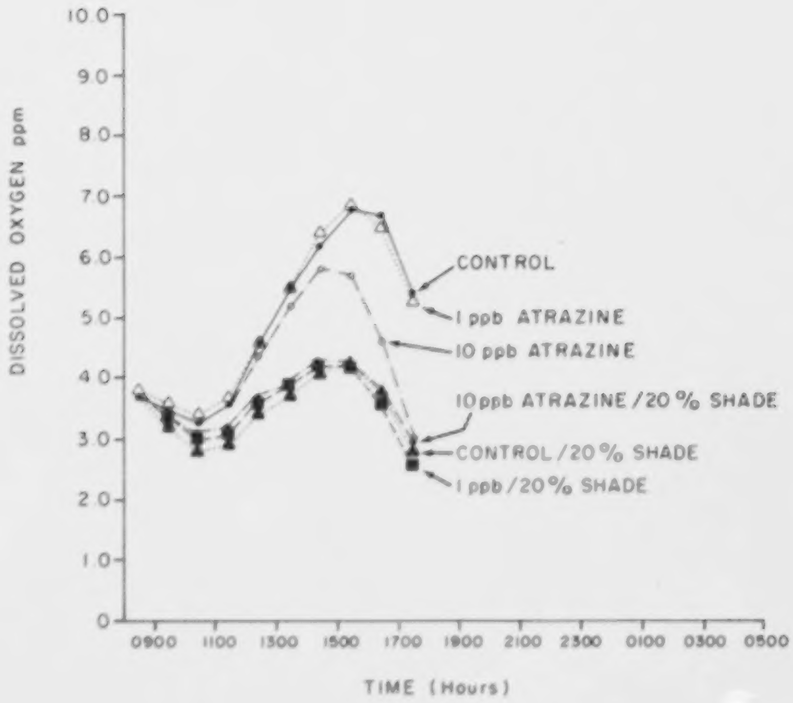

Figure C5.16. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 9 September 1980, involving control, shaded control (20%), 1 ppb atrazine, shaded 1 ppb atrazine (20%), 10 ppb atrazine and shaded 10 ppb atrazine (20%) domes. Refer to Tables 5.16 (DO vs. Time) and 5.34 (mg O_2 m^{-2} hr^{-1} vs. Time).

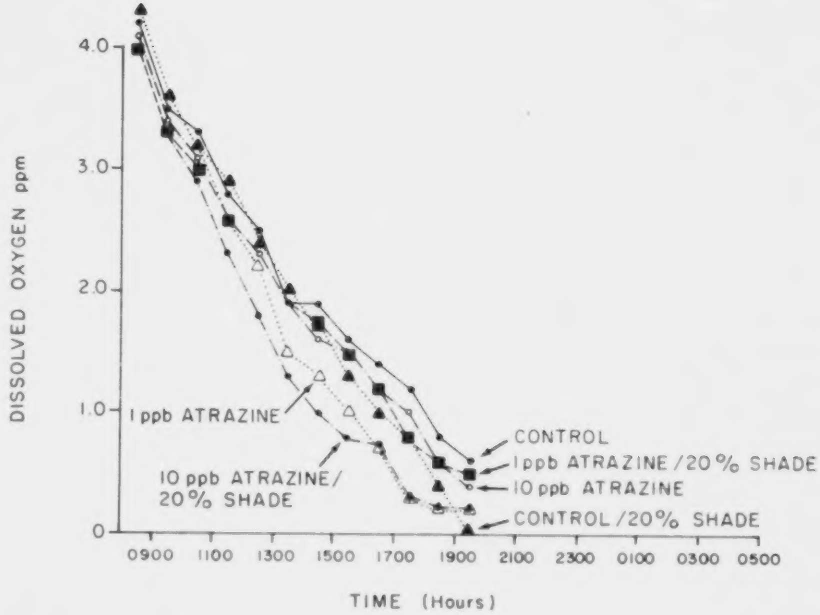

Figure C5.17. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 10 September 1980, involving control, shaded control (20%), 1 ppb atrazine, shaded 1 ppb atrazine (20%), 10 ppb atrazine and shaded 10 ppb atrazine (20%) domes. Refer to Tables 5.17 (DO vs. Time) and 5.35 (mg O_2 m^{-2} hr^{-1} vs. Time).

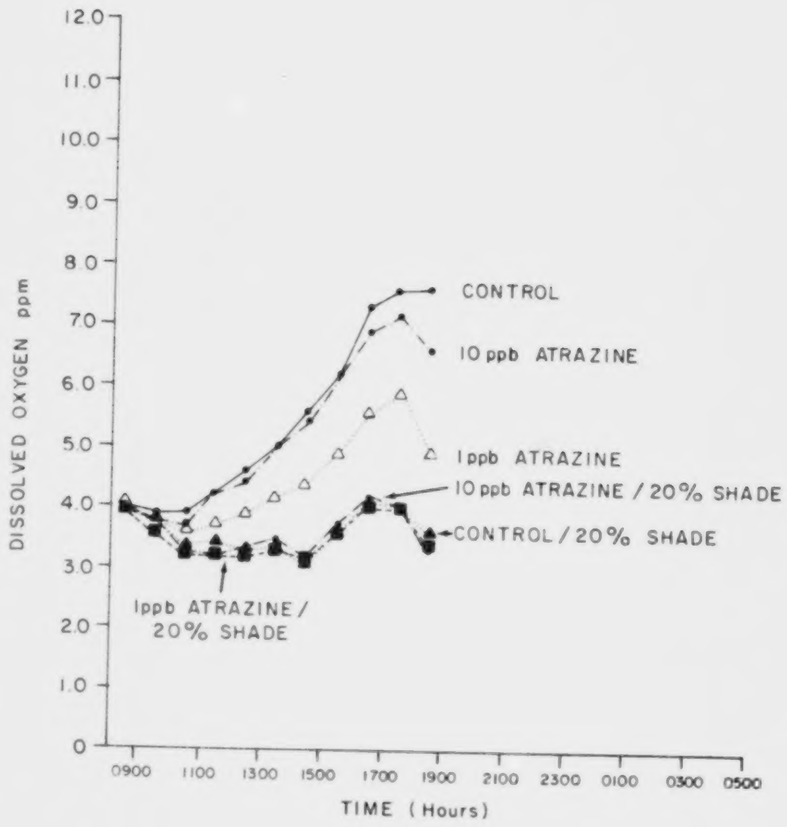

Figure C5.18. Dissolved oxygen (ppm) versus time (hours). Guinea Marsh dome study, 11 September 1980, involving control, shaded control (20%), 1 ppb atrazine, shaded 1 ppb atrazine (20%), 10 ppb atrazine and shaded 10 ppb atrazine (20%) domes. Refer to Tables 5.18 (DO vs. Time) and 5.36 (mg O_2 m^{-2} hr^{-1} vs. Time).

TABLE C5.19. GUINEA MARSH DOME SET, 29-30 MAY 1980

Date	Time*	mg O_2 m^{-2} hr^{-1}			
		Control	MEOH	100 ppb** Light	100 ppb** Dark
5/29/80	1730	-200.00	-200.00	133.33	-366.67
	1830	-366.67	-333.33	-233.33	-200.00
	1930	-233.33	-333.33	-200.00	-266.67
	2030	-400.00	-566.67	-400.00	-366.67
	2130	166.67	-100.00	-166.67	-166.67
	2230	-300.00	-566.67	-200.00	-300.00
	2330	-133.33	-333.33	-400.00	-100.00
5/30/80	0030	-200.00	66.67	0.0	-200.00
	0130	-266.67	-266.67	-400.00	-400.00
	0300	-133.33	- 83.33	- 83.33	- 33.33
	0430	266.67	166.67	100.00	100.00
	0530	- 66.67	-133.33	-200.00	-200.00
	0630	200.00	33.33	-133.33	-100.00
	0730	166.67	- 33.33	-133.33	0.0
	0830	166.67	200.00	166.67	0.0
	0930	533.33	400.00	133.33	66.67
	1030	200.00	333.33	166.67	66.67
	1130	66.67	66.67	- 66.67	-133.33
	1230	1500.00	1100.00	533.33	66.67
	1330	200.00	300.00	100.00	0.0
	1430	166.67	333.33	100.00	0.0
	1530	200.00	200.00	66.67	0.0
	1630	0.0	0.0	33.33	66.67

* Time = midpoint of hour, e.g. 0700-0800
** Nominal dissolved atrazine concentrations

TABLE C5.20. GUINEA MARSH DOME SET, 23-24 JUNE 1980

Date	Time*	$mg\ O_2\ m^{-2}\ hr^{-1}$			
		Control	MEOH	10 ppb**	100 ppb**
6/23/80	0730	133.33	200.00	200.00	- 33.33
	0830	266.67	366.67	200.00	266.67
	0930	766.67	866.67	566.67	33.33
	1030	200.00	266.67	333.33	0.0
	1130	266.67	400.00	333.33	0.0
	1230	200.00	266.67	233.33	- 66.67
	1330	33.33	100.00	100.00	33.33
	1430	66.67	33.33	33.33	-100.00
	1530	- 66.67	-133.33	-266.67	-400.00
	1630	-300.00	-300.00	4466.67	-300.00
	1730	-233.33	-366.67	-5400.00	-433.33
	1830	-566.67	-666.67	-533.33	-300.00
	1930	-333.33	-466.67	-500.00	-300.00
	2030	-500.00	-600.00	-433.33	-150.00
	2130	-466.67	-500.00	-500.00	- 16.67
	2230	—	—	—	—
	2330	233.33	-377.78	-238.89	0.0
6/24/80	0130	-1450.00	-150.00	-116.67	- 16.67
	0230	- 50.00	- 66.67	- 33.33	0.0
	0330	-116.67	33.33	- 50.00	0.0
	0430	- 16.67	- 16.67	0.0	0.0
	0530	33.33	50.00	16.67	16.67
	0630	0.0	- 33.33	33.33	0.0
	0730	16.67	83.33	233.33	0.0
	0830	116.67	233.33	366.67	0.0

* Time = midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.21. GUINEA MARSH DOME SET, 25-26 JUNE 1980

Date	Time*	mg O_2 m^{-2} hr^{-1}					
		Control	MEOH	1 ppb**	10 ppb**	100 ppb**	1000 ppb**
6/25/80	0830	266.67	200.00	233.33	200.00	133.33	100.00
	0930	166.67	166.67	133.33	167.67	-100.00	-200.00
	1030	300.00	266.67	233.33	233.33	100.00	- 66.67
	1130	366.67	266.67	366.67	366.67	100.00	- 66.67
	1230	266.67	366.67	300.00	300.00	66.67	0.0
	1330	133.33	100.00	100.00	200.00	- 66.67	-166.67
	1430	-100.00	166.67	0.0	-100.00	-166.67	-133.67
	1530	-266.67	-333.33	-266.67	-300.00	-333.33	-300.00
	1630	-266.67	-133.33	-266.67	-266.67	-433.33	-266.67
	1730	-433.33	-400.00	-466.67	-433.33	-366.67	-166.67
	1830	-500.00	-433.33	-533.33	-466.67	-333.33	-166.67
	1930	-466.67	-366.67	-500.00	-466.67	- 50.00	-216.67
	2030	-433.33	--	- 83.33	-366.67	-266.67	--
	2200	-300.00	-408.33	-383.33	-258.33	- 16.67	- 25.00
	2330	-166.67	-600.00	- 50.00	-200.00	- 16.67	- 16.67
6/26/80	0030	0.0	- 66.67	0.0	- 33.33	0.0	16.67
	0130	0.0	0.0	- 33.33	- 33.33	- 16.67	0.0
	0230	0.0	- 33.33	0.0	16.67	0.0	0.0
	0400	25.00	- 50.00	0.0	8.33	125.00	0.0
	0530	- 83.33	- 16.67	0.0	- 33.33	-250.00	- 33.33
	0630	0.0	216.67	50.00	716.67	416.67	16.67
	0730	16.67	-133.33	0.0	-700.00	-416.67	66.67

* Time = midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.22. GUINEA MARSH DOME SET, 15-16 JULY 1980

		mg O_2 m^{-2} hr^{-1}					
Date	Time*	Control	MEOH	1 ppb**	10 ppb**	100 ppb**	1000 ppb**
7/15/80	0730	733.33	500.00	566.67	266.67	433.33	133.33
	0830	-433.33	0.0	66.67	533.33	100.00	-733.33
	0930	900.00	566.67	633.33	500.00	233.33	533.33
	1030	133.33	333.33	366.67	600.00	233.33	-1100.00
	1130	700.00	400.00	266.67	466.67	300.00	- 66.67
	1230	466.67	533.33	466.67	433.33	200.00	-166.67
	1330	366.67	366.67	366.67	433.33	66.67	-66.67
	1430	300.00	333.33	300.00	266.67	133.33	- 83.33
	1530	200.00	266.67	200.00	266.67	0.0	-116.67
	1630	- 33.33	66.67	66.67	- 66.67	-100.00	33.33
	1730	- 33.33	--	- 66.67	-133.33	-266.67	-266.67
	1830	-533.33	-266.67	-400.00	-466.67	-466.67	- 66.67
	1930	-666.67	-466.67	-416.67	-600.00	-600.00	- 33.33
	2030	-633.33	-533.33	-566.67	-633.33	-566.67	0.0
	2130	-666.67	-566.67	-733.33	-633.33	-500.00	0.0
	2230	-600.00	-533.33	-566.67	-600.00	-416.67	0.0
	2330	-600.00	-633.33	-600.00	-666.67	-216.67	0.0
7/16/80	0030	-566.67	-516.67	-566.67	-583.33	- 66.67	0.0
	0130	-433.33	-433.33	-433.33	-450.00	- 33.33	- 16.67
	0230	-250.00	-350.00	-316.67	-383.33	0.0	0.0
	0330	-166.67	-266.67	-250.00	- 83.33	- 16.67	0.0
	0430	-100.00	-183.33	-133.33	- 16.67	0.0	0.0
	0530	16.67	- 66.67	- 16.67	0.0	0.0	0.0
	0630	16.67	0.0	16.67	0.0	0.0	- 10.00
	0730	233.33	233.33	183.33	100.00	0.0	10.00
	0830	416.67	333.33	300.00	200.00	16.67	0.0
	0930	366.67	283.33	250.00	150.00	0.0	0.0
	1030	366.67	333.33	283.33	200.00	0.0	0.0
	1130	433.33	350.00	333.33	200.00	16.67	0.0
	1230	533.33	416.67	383.33	283.33	0.0	16.67
	1330	466.67	333.33	300.00	250.00	0.0	0.0

* Time = midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.23. GUINEA MARSH DOME SET, 14 JULY 1980

		mg O_2 m^{-2} hr^{-1}					
Date	Time*	Control	MEOH	1 ppb**	10 ppb**	100 ppb**	1000 ppb**
7/14/81	0800	233.33	166.67	300.00	400.00	400.00	-200.00
	0900	400.00	466.67	433.33	600.00	133.33	-333.33
	1000	433.33	433.33	466.67	600.00	200.00	-300.00
	1100	400.00	300.00	400.00	733.33	133.33	-216.67
	1200	400.00	133.33	400.00	166.67	133.33	-166.67
	1300	400.00	66.67	400.00	366.67	200.00	-150.00
	1400	200.00	100.00	200.00	166.67	266.67	- 83.33
	1500	166.67	-200.00	133.33	- 33.33	-333.33	-100.00
	1600	133.33	-166.67	0.0	-167.67	-166.67	- 50.00
	1700	-200.00	-366.67	-266.67	-400.00	-333.33	0.0

* Time = midpoint of hour, e.g. 0700-0800
** Nominal dissolved atrazine concentrations

TABLE C5.24. GUINEA MARSH DOME SET, 18-19 JULY 1980

		$mg\ O_2\ m^{-2}\ hr^{-1}$					
Date	Time*	Control	MEOH	1 ppb**	10 ppb**	100 ppb**	1000 ppb**
7/18/80	0730	100.00	100.00	166.67	33.33	166.67	-200.00
	0830	233.33	366.67	300.00	233.33	233.33	-233.33
	0930	366.67	433.33	333.33	266.67	233.33	-200.00
	1030	200.00	283.33	200.00	66.67	66.67	-166.67
	1130	333.33	200.00	300.00	200.00	100.00	-133.33
	1230	433.33	633.33	433.33	300.00	233.33	-100.00
	1330	500.00	400.00	400.00	233.33	166.67	- 33.33
	1430	300.00	333.33	233.33	166.67	66.67	0.0
	1530	200.00	200.00	200.00	0.0	- 66.67	-33.33
	1630	- 66.67	0.0	- 33.33	-133.33	-133.33	0.0
	1730	-266.67	-233.33	-366.67	-433.33	-366.67	0.0
	1830	-333.33	-366.67	-533.33	-600.00	-366.67	0.0
	1930	-633.33	-633.33	-633.33	-650.00	-450.00	- 16.67
	2030	-566.67	-600.00	-800.00	-600.00	-450.00	- 16.67
	2130	-783.33	-816.67	-766.67	-183.33	-366.67	- 16.67
	2230	-316.67	-450.00	-400.00	0.0	-133.33	0.0
	2330	-100.00	-533.33	- 50.00	- 16.67	- 16.67	0.0
7/19/80	0030	-433.33	-250.00	- 83.33	0.0	0.0	0.0
	0130	-133.33	- 66.67	0.0	0.0	0.0	0.0
	0230	- 16.67	16.67	0.0	0.0	0.0	0.0
	0330	- 16.67	- 33.33	0.0	0.0	0.0	0.0
	0430	- 16.67	0.0	0.0	0.0	0.0	0.0
	0530	0.0	16.67	0.0	16.67	0.0	0.0

* Time = Midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.25. GUINEA MARSH DOME SET, 29 JULY 1980

Date	Time*	mg O_2 m^{-2} hr^{-1}					
		Control	Control 51% Shade	MEOH	MEOH 51% Shade	10 ppb**	10 ppb** 51% Shade
7/29/80	0730	-126.67	-266.67	-230.00	- 46.67	-233.33	-290.00
	0830	- 50.00	-113.33	- 56.67	-126.67	- 60.00	-130.00
	0930	183.33	16.67	173.33	23.33	203.33	0.0
	1030	383.33	133.33	413.33	133.33	420.00	103.33
	1130	166.67	- 23.33	150.00	- 33.33	116.67	- 36.67
	1230	393.33	90.00	0.0	106.67	343.33	210.00
	1330	210.00	0.0	580.00	- 3.33	156.67	-143.33
	1430	306.67	13.33	296.67	0.0	260.00	16.67
	1530	173.33	- 80.00	143.33	-116.67	- 40.00	-116.67
	1630	20.00	-116.67	- 86.67	-180.00	-126.67	-113.33
	1800	-111.67	- 85.00	-148.33	- 66.33	-541.67	- 66.67
	1930	-1036.67	- 36.67	-1210.00	- 0.67	-386.67	10.00
	2030	-326.67	26.67	-286.67	21.67	-156.67	10.00
	2130	-430.00	23.33	-270.00	11.67	- 20.00	- 6.67

* Time = Midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.26. GUINEA MARSH DOME SET, 30 JULY 1980

Date	Time*	mg O_2 m^{-2} hr^{-1}					
		Control	Control 51% Shade	MEOH	MEOH 51% Shade	10 ppb**	10 ppb** 51% Shade
7/30/80	0800	30.00	-230.00	13.33	-266.67	- 3.33	-150.00
	0900	276.67	-100.00	410.00	-136.67	126.67	-130.00
	1000	286.67	- 20.00	313.33	- 60.00	193.33	- 30.00
	1100	346.67	56.67	336.67	40.00	200.00	13.33
	1200	370.00	93.33	340.00	63.33	256.67	80.00
	1300	443.33	86.67	360.00	40.00	266.67	253.33
	1400	- 43.33	- 33.33	- 53.33	- 10.00	120.00	-150.00
	1500	303.33	60.00	196.67	60.00	123.33	- 20.00
	1600	156.67	- 46.67	126.67	-110.00	103.33	- 90.00
	1700	-193.33	-280.00	-256.67	-153.33	-270.00	-190.00
	1745	-580.00	-280.00	-933.33	-126.67	-1306.67	-433.33

* Time = Midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.27. GUINEA MARSH DOME SET, 31 JULY 1980

Date	Time*	$mg\ O_2\ m^{-2}\ hr^{-1}$ Control	Control 51% Shade	MEOH	MEOH 51% Shade	10 ppb**	10 ppb** 51% Shade
7/30/80	0800	73.33	466.67	50.00	-120.00	3.33	-236.67
	0900	286.67	830.00	303.33	-330.00	156.67	- 43.33
	1000	293.33	470.00	460.00	- 20.00	216.67	- 40.00
	1100	280.00	203.33	940.00	23.33	163.33	3.33
	1200	1073.33	66.67	-836.67	- 66.67	76.67	- 43.33
	1300	-663.33	146.67	143.33	- 20.00	76.67	- 40.00
	1400	80.00	96.67	- 80.00	340.00	443.33	320.00
	1500	273.33	723.33	-217.80	-284.40	-510.00	173.33
	1545	-800.00	-1700.00	--	--	-880.00	-1033.33
	1630	-100.00	--	-300.00	-116.67	-233.33	0.0
	1730	-433.33	--	-766.67	- 33.33	600.00	16.67
	1830	1233.33	--	-500.00	- 16.67	-1350.00	- 33.33
	1930	-2350.00	--	- 16.67	- 16.67	- 16.67	- 16.67

* Time = midpoint of hour, e.g. 0700-0800
** Nominal dissolved atrazine concentrations

TABLE C5.28. GUINEA MARSH DOME SET, 1 AUGUST 1980

Date	Time*	mg O_2 m^{-2} hr^{-1}			
		Control	Control 30% Shade	10 ppb**	10 ppb** 30% Shade
8/1/80	0830	100.00	-186.67	116.67	-320.00
	0930	126.67	- 63.33	200.00	- 56.67
	1030	220.00	33.33	130.00	- 56.67
	1130	153.33	- 33.33	60.00	-130.00
	1230	163.33	- 43.33	16.67	-166.67
	1330	123.33	- 20.00	- 10.00	-106.67
	1430	113.33	- 86.67	- 90.00	-163.33

* Time = Midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.29. GUINEA MARSH DOME SET, 12 AUGUST 1980

Date	Time*	mg O_2 m^{-2} hr^{-1} Control	Control 30% Shade	1 ppb**	1 ppb** 30% Shade	10 ppb**	10 ppb** 30% Shade
8/12/80	0900	-453.33	-426.67	-333.33	-523.33	-346.67	-676.67
	1000	-166.67	-203.33	-103.33	-270.00	-110.00	-333.33
	1100	3.33	-123.33	- 3.33	-116.67	23.33	-176.67
	1200	76.67	- 73.33	113.33	- 30.00	100.00	- 73.33
	1300	60.00	- 80.00	90.00	- 63.33	43.33	- 80.00
	1400	100.00	- 30.00	96.67	6.67	90.00	26.67
	1500	243.33	156.67	296.67	153.33	280.00	150.00
	1600	30.00	6.67	126.67	- 16.67	53.33	13.33
	1700	-153.33	-193.33	- 96.67	-180.00	-143.33	-126.67
	1800	-430.00	-320.00	-356.67	-320.00	-410.00	-246.67
	1900	-540.00	-266.67	-553.33	-206.67	-513.33	-153.33

* Time = Midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.30. GUINEA MARSH DOME SET, 13 AUGUST 1980

		$mg\ O_2\ m^{-2}\ hr^{-1}$					
Date	Time*	Control	Control 30% Shade	1 ppb**	1 ppb** 30% Shade	10 ppb**	10 ppb** 30% Shade
8/13/80	0800	190.00	-206.67	-163.33	-173.33	150.00	10.00
	0900	713.33	100.00	1000.00	120.00	723.33	360.00
	1000	200.00	66.67	200.00	0.0	166.67	50.00
	1100	500.00	116.67	333.33	233.33	433.33	133.33
	1200	366.67	383.33	866.67	400.00	733.33	316.67
	1300	466.67	200.00	200.00	166.67	333.33	166.67
	1400	350.00	200.00	833.33	200.00	616.67	200.00
	1500	383.33	66.67	233.33	0.0	183.33	- 33.33
	1600	200.00	-200.00	66.67	-166.67	66.67	-100.00
	1700	-533.33	-583.33	-466.67	-583.33	-466.67	-466.67
	1800	-766.67	-516.67	-600.00	-583.33	-633.33	-616.67
	1900	-1333.33	-416.67	-1033.33	-416.67	-1033.33	-633.33

* Time = Midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.31. GUINEA MARSH DOME SET, 14 AUGUST 1980

		mg O_2 m^{-2} hr^{-1}					
Date	Time*	Control	Control 30% Shade	1 ppb**	1 ppb** 30% Shade	10 ppb**	10 ppb** 30% Shade
8/14/80	0800	0.00	-233.33	- 50.00	-233.33	-166.67	-300.00
	0900	- 33.33	-200.00	- 33.33	-166.67	- 66.67	-200.00
	1000	100.00	- 33.33	133.33	0.00	100.00	- 50.00
	1100	250.00	166.67	166.67	233.33	200.00	133.33
	1200	266.67	166.67	216.67	200.00	183.33	133.33
	1300	283.33	66.67	66.67	83.33	66.67	16.67
	1400	-166.67	-150.00	-250.00	-250.00	-366.67	-266.67
	1500	33.33	-116.67	-266.67	-233.33	-283.33	0.00
	1600	-266.67	-100.00	-283.33	-250.00	-366.67	-383.33
	1700	-333.33	-283.33	-483.33	--	--	--
	1800	-400.00	- 83.33	-191.67	-156.56	-144.44	- 58.33
	1900	-400.00	--	--	--	--	- 50.00
	2000	-383.33	- 83.33	0.00	- 16.67	0.00	16.67
	2100	0.00	- 16.67	33.33	0.00	0.00	0.00
	2200	- 16.67	- 16.67	- 50.00	0.00	- 16.67	- 16.67

* Time = Midpoint of hour, e.g. 0700-0800
** Nominal dissolved atrazine concentrations

TABLE C5.32. GUINEA MARSH DOME SET, 15 AUGUST 1980

Date	Time*	mg O_2 m^{-2} hr^{-1}					
		Control	Control 30% Shade	1 ppb**	1 ppb** 30% Shade	10 ppb**	10 ppb** 30% Shade
8/15/80	0800	-120.00	-466.67	-300.00	-550.00	-550.00	-516.67
	0900	33.33	-266.67	-166.67	-333.33	-233.33	-333.33
	1000	133.33	-100.00	-100.00	-200.00	- 83.33	-166.67
	1100	233.33	- 50.00	- 16.67	- 16.67	16.67	- 50.00
	1200	100.00	- 33.33	-116.67	--	- 16.67	- 66.67
	1300	33.33	- 66.67	-133.33	- 25.00	-133.33	- 83.33
	1400	66.67	- 50.00	-150.00	-133.33	-116.67	- 83.33
	1500	-100.00	-100.00	-100.00	- 50.00	- 50.00	- 16.67
	1600	- 66.67	-100.00	- 66.67	- 50.00	-100.00	- 83.33

* Time - Midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.33. GUINEA MARSH DOME SET, 8 SEPTEMBER 1980

Date	Time*	mg O_2 m^{-2} hr^{-1}					
		Control	Control 20% Shade	1 ppb**	1 ppb** 20% Shade	10 ppb**	10 ppb** 20% Shade
9/8/80	0900	33.33	-100.00	- 33.33	- 66.67	133.33	66.67
	1000	266.67	33.33	233.33	- 66.67	200.00	333.33
	1100	280.00	120.00	280.00	320.00	360.00	160.00
	1200	400.00	142.86	428.57	114.29	342.86	285.71
	1300	400.00	266.67	400.00	166.67	633.33	366.67
	1400	333.33	200.00	300.00	100.00	266.67	333.33
	1500	- 33.33	-100.00	- 33.33	-233.33	66.67	- 66.67
	1600	0.00	-200.00	-100.00	-200.00	- 66.67	-133.33
	1700	-266.67	-233.33	-400.00	-366.67	-333.33	-366.67
	1800	-400.00	-266.67	-466.67	-300.00	-466.67	-466.67
	1900	-566.67	-466.67	-600.00	-233.33	-500.00	-600.00

* Time - Midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.34. GUINEA MARSH DOME SET, 9 SEPTEMBER 1980

Date	Time*	$mg\ O_2\ m^{-2}\ hr^{-1}$					
		Control	Control 20% Shade	1 ppb**	1 ppb** 20% Shade	10 ppb**	10 ppb** 20% Shade
9/9/80	0800	- 66.67	-166.67	- 66.67	-133.33	- 33.33	-133.33
	0900	- 66.67	-133.33	- 66.67	-133.33	-100.00	-100.00
	1000	100.00	33.33	100.00	33.33	100.00	33.33
	1100	333.33	166.67	300.00	166.67	266.67	166.67
	1200	300.00	100.00	300.00	100.00	266.67	66.67
	1300	233.33	133.33	300.00	100.00	200.00	133.33
	1400	200.00	66.67	166.67	0.00	- 33.33	0.00
	1500	- 33.33	-166.67	-133.33	-200.00	-366.67	-166.67
	1600	-433.33	-333.33	-400.00	-333.33	-500.00	-266.67

* Time = Midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

TABLE C5.35. GUINEA MARSH DOME SET, 10 SEPTEMBER 1980

Date	Time*	$mg\ O_2\ m^{-2}\ hr^{-1}$					
		Control	Control 20% Shade	1 ppb**	1 ppb** 20% Shade	10 ppb**	10 ppb** 20% Shade
9/10/80	0800	-233.33	-233.33	-200.00	-233.33	-233.33	-266.67
	0900	- 66.67	-133.33	-133.33	-100.00	-100.00	-133.33
	1000	-166.67	-100.00	-200.00	- 66.67	-166.67	-200.00
	1100	-120.00	-200.00	-160.00	-120.00	-120.00	-200.00
	1200	-200.00	-114.29	-200.00	-171.43	-114.29	-142.86
	1300	0.00	-100.00	- 66.67	- 33.33	- 66.67	-100.00
	1400	- 66.67	-133.33	-100.00	-100.00	- 66.67	- 66.67
	1500	- 66.67	-100.00	-100.00	-100.00	-100.00	-100.00
	1600	- 66.67	- 66.67	-133.33	-133.33	- 66.67	- 66.67
	1700	-133.33	-133.33	- 33.33	- 66.67	-100.00	- 33.33
	1800	- 66.67	- 66.67	0.00	- 33.33	-100.00	0.00

* Time = Midpoint of hour, e.g. 0700-0800
** Nominal dissolved atrazine concentrations

TABLE C5.36. GUINEA MARSH DOME SET, 11 SEPTEMBER 1980

Date	Time*	mg O_2 m^{-2} hr^{-1}					
		Control	Control 20% Shade	1 ppb**	1 ppb** 20% Shade	10 ppb**	10 ppb** 20% Shade
9/11/80	0800	- 33.33	- 66.67	-100.00	-133.33	- 33.33	- 66.67
	0900	0.00	-133.33	- 66.67	-133.33	- 66.67	-166.67
	1000	100.00	0.00	33.33	0.00	166.67	- 33.33
	1100	133.33	- 33.33	66.67	0.00	66.67	33.33
	1200	133.33	0.00	100.00	33.33	200.00	66.67
	1300	200.00	- 66.67	66.67	- 33.33	133.33	-100.00
	1400	200.00	166.67	166.67	133.33	233.33	200.00
	1500	366.67	166.67	233.33	133.33	266.67	133.33
	1600	100.00	566.67	66.67	0.00	100.00	- 66.67
	1700	0.00	-733.33	-333.33	-200.00	-200.00	-200.00

* Time - Midpoint of hour, e.g. 0700-0800

** Nominal dissolved atrazine concentrations

SECTION 6

GREENHOUSE STUDIES

INTRODUCTION

The greenhouse experiments were designed to accomplish longer term exposures of Zostera marina to atrazine than we could accomplish with field experiments.

The results of the field surveys, particularly the Severn River survey, indicated herbicides were carried into the estuary by runoff and subsequently be found subsequently in the water over SAV beds for periods of several days. In an effort to evaluate the potential effects a long-term, low-level exposure to atrazine might produce in Zostera, we undertook a series of three week chronic dosing experiments. The three week period was selected to be longer than we believed a typical exposure in the lower Chesapeake Bay might be. (This was based on sampling in the Severn River system, and a general assumption about flushing times in other subestuaries.) The dosage levels were the same as those used in the dome studies. The range of concentrations was suggested by our 1978 survey of concentrations in the lower Chesapeake Bay.

METHODS

Zostera marina plants collected from the lower York River were exposed to atrazine in a flow through dosing system. The aboveground morphology of the plants was monitored in an effort to detect effects of the exposure.

The dosing apparatus (see Figure 6.1) utilized 37.8 liter glass aquaria as test chambers. Water from the York River at Gloucester Point (approximately 20 ppt salinity) was pumped into the greenhouse and filtered by 10 Gaflo (trade name) polypropelene bag filters. Filtered water was collected in a storage tank from which it was continuously pumped to a constant level header tank. Calibrated siphons delivered the water to individual glass mixing chambers. Stock solutions of atrazine (in either methanol or acetone) were also delivered to the mixing chambers by a peristaltic pump. The water with the added atrazine was then delivered by glass tubing to a glass flow splitter which was designed as a secondary header tank. Calibrated siphons delivered the water-herbicide to duplicate dosing tanks for each test concentration. Water entered the top rear of each dosing tank and exited from the bottom front by a constant prime siphon. The peristaltic pump was connected to a float switch which prevented dosing if diluent water flow ceased.

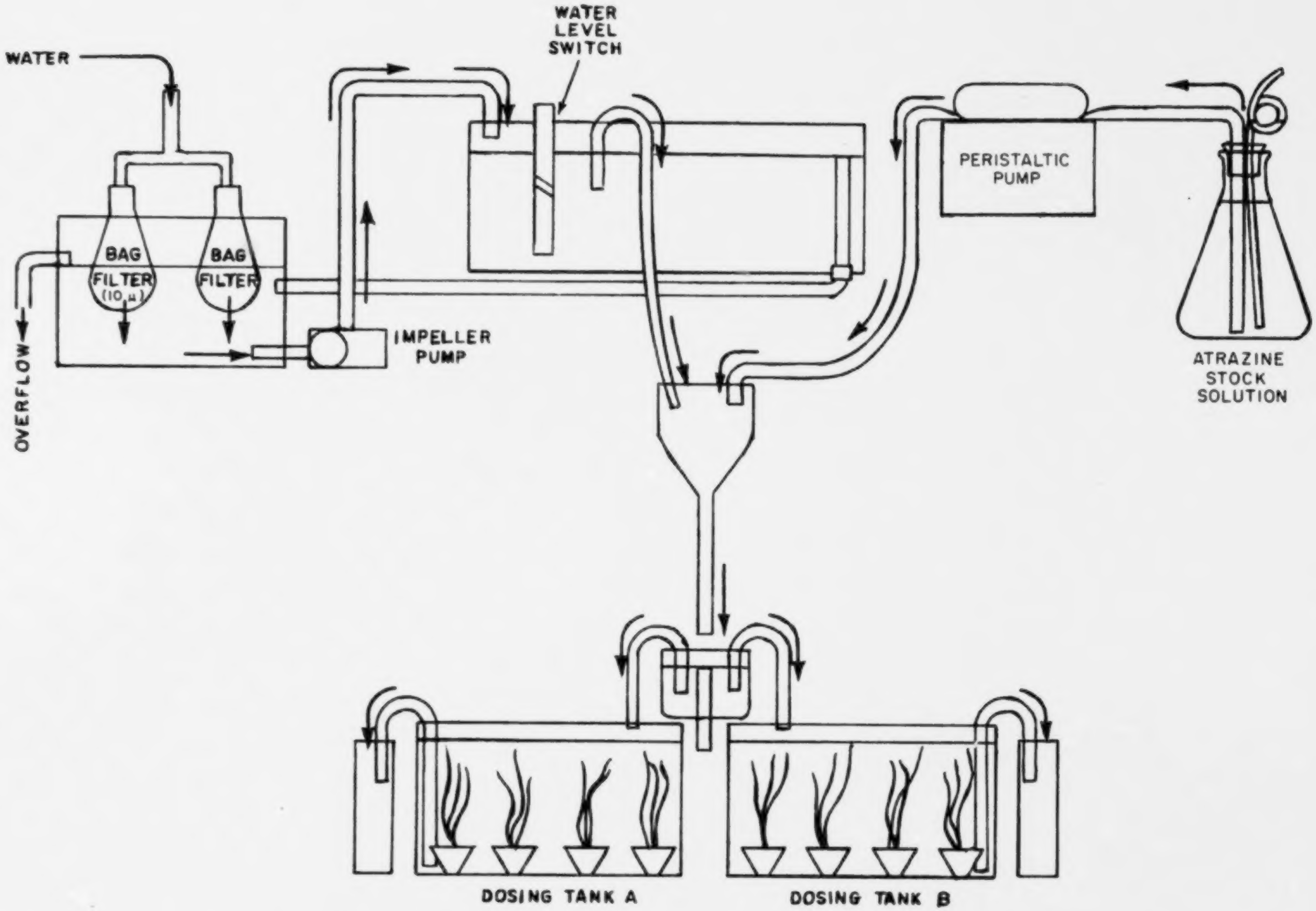
WATER
WATER LEVEL SWITCH
BAG FILTER (10 μ)
BAG FILTER
OVERFLOW
IMPELLER PUMP
PERISTALTIC PUMP
ATRAZINE STOCK SOLUTION
DOSING TANK A
DOSING TANK B

Figure 6.1 Long-term dosing apparatus. The diagram depicts only two of the twelve tanks in the system. Each pair of tanks has a separate mixing chamber and constant flow siphons.

The entire system was allowed to fill with the appropriate atrazine concentration prior to initiation of an experiment. The flow rates of all calibrated siphons and the toxicant delivery rates were monitored daily. Maximum and minimum water temperatures were also monitored daily, although no effort is made to regulate them. No effort was made to regulate the photoperiod. A 50% shading cloth was placed over the greenhouse during summer months to prevent photoinhibition and to help minimize inside air temperatures.

Each experiment utilized fifteen individually potted plants per dosing tank. The plants were placed in small peat pots filled with subtidal mud. Plants were measured at the beginning of each experiment and, depending on the experiment, at weekly intervals or at the end of the dosing. Each plant was measured for the height of the longest leaf, total number of leaves, and total number of shoots. A shoot was defined as any leaf group separated by more than one centimeter from other groups. All plants were harvested, rinsed, and divided into aboveground and belowground tissues at the termination of an experiment. Plant tissues were pooled for each dosage tank and subjected to analysis for atrazine content.

The data from each experiment was analyzed by calculating a mean percent change in the test parameters over the course of the experiment. The mean percent change was based on the initial measurements, and calculated as

$$\text{mean percent change} = \frac{X_A - X_0 \ (100)}{X_0}$$

where: X_0 = mean of parameter at time zero
X_A = mean of parameter after time A

This index varies between +100% and -100% with 0 indicating no change over the time interval. A -50% value indicates a 50% reduction in the parameter measured. Twenty one day LC_{50}'s and EC_{50}'s for each test parameter were determined by the graphic method. Dead plants were not included in the data analyses used to determine the EC_{50}'s.

RESULTS

The data for experiments conducted in 1980 are reported in the appendix to this section, Tables D6.1 through D6.28. Experiment 5 (Tables D6.14 through D6.20) is omitted from further data analysis because of the unacceptable mortality in control treatments.

A twenty-one day LC_{50} was determined by the graphic method to be 0.07 mgl^{-1} (70 ppb). Data from the experiments were pooled for this analysis (see Figure 6.2).

The effect of atrazine on plant height, number of leaves, and number of shoots is graphed in Figure 6.3. Again data from all the experiments were pooled for this analysis. The EC_{50} for atrazine effects on plant height was 0.41 mgl^{-1} (410 ppb). The EC_{50} for atrazine effects on number of leaves was 0.06 mgl^{-1} (60 ppb). The EC_{50} for atrazine effects on number of shoots was

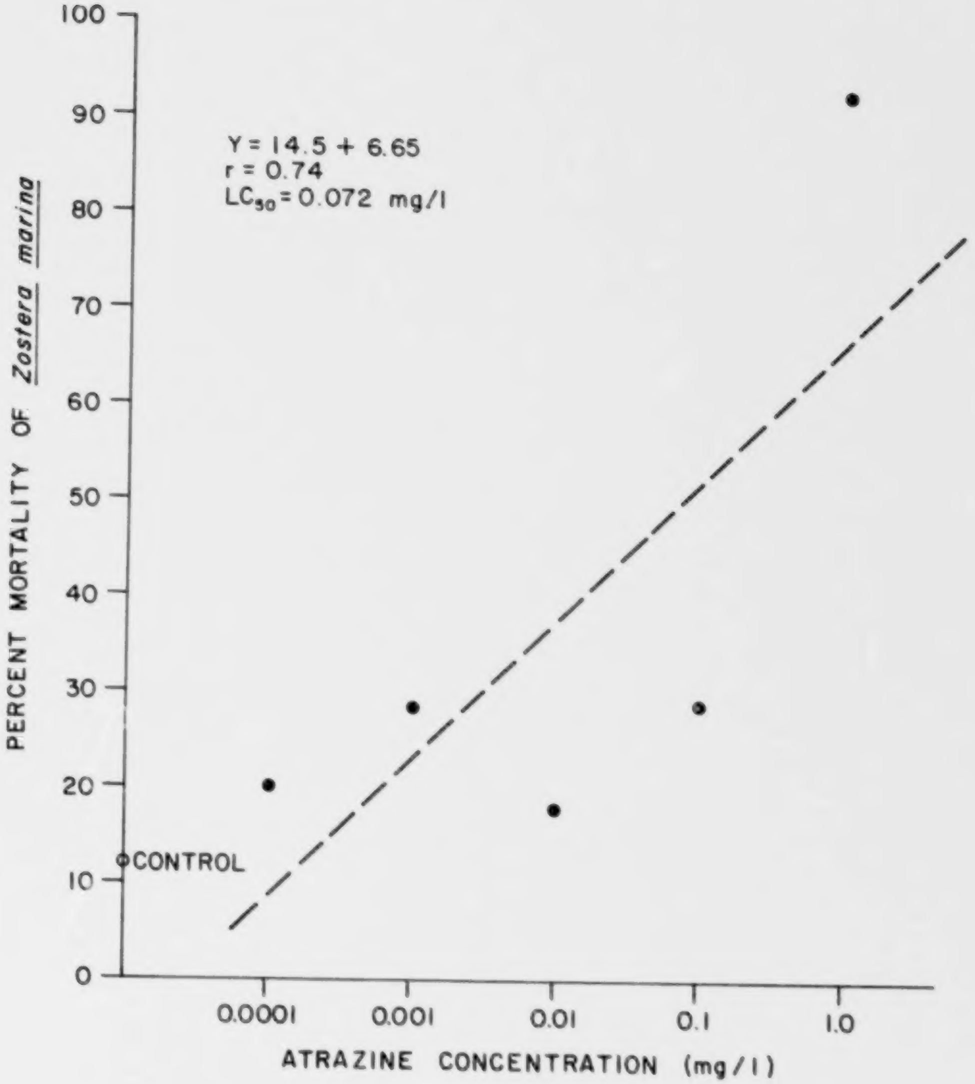

Figure 6.2. Graph of percent Zostera mortality in test chambers vs. atrazine concentration. Linear regression line is plotted, LC_{50} determined by extrapolation using the regression equation.

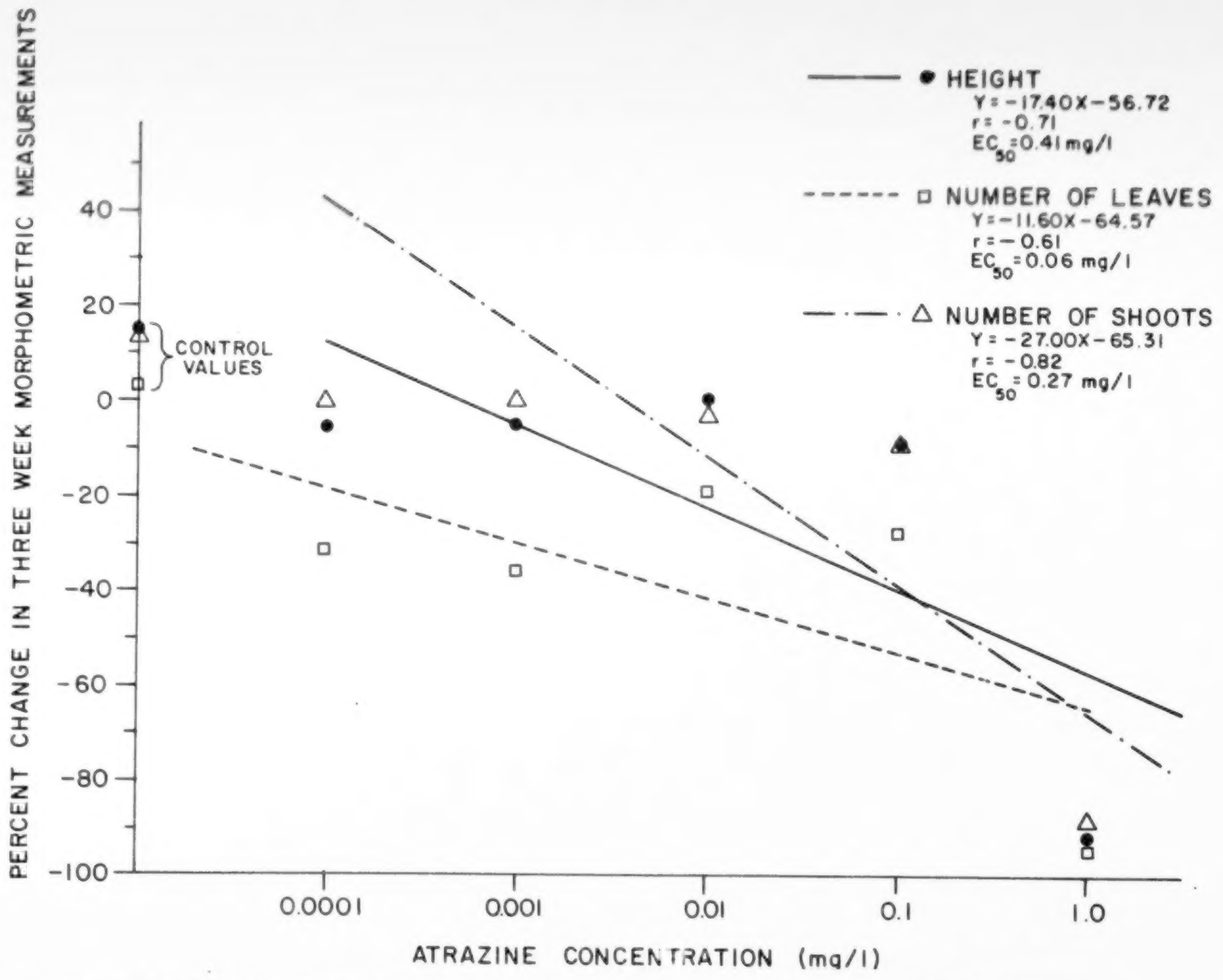

Figure 6.3. Graph of percent change in three week morphometric measurements vs. atrazine concentration (mgl^{-1}). Morphometric measurements include height (circles and solid regression line), number of leaves (squares and dashed regression line), and number of shoots (triangles and dot-dash regression line).

0.27 mgl^{-1} (270 ppb). Confidence limits for these values have not been developed due to the highly variable nature of the morphometric data.

The effects of the six atrazine concentrations on the morphological parameters through time are graphed in Figures 6.4, 6.5 and 6.6. Mean height of the Zostera plants was decreased 50% during the test period by only the highest concentration, 1.0 mgl^{-1} (1000 ppb). All concentrations except the control and the 0.1 mgl^{-1} (100 ppb) level produced negative slopes for linear regression lines fitted to the data. The 0.1 mgl^{-1} (100 ppb) data produced a regression slope of 0.314. This positive slope appears to be caused by the marked reduction in height recorded on day 16. The only clearcut effect of atrazine on plant height was achieved by the 1.0 mgl^{-1} (1000 ppb) concentration which produced a 50% reduction in mean height within approximately 14 days.

The effect of atrazine on the mean number of leaves per plant was similar to the effects on mean height. Linear regression analyses demonstrated that the 1.0 mgl^{-1} concentration (1000 ppb) produced the most marked effects, resulting in a 50% reduction in number of leaves within approximately 13 days. Other concentrations also produced a decrease in leaf number, according to the regression analysis, but none effected a 50% decrease in numbers within the test period.

The number of shoots per plant was reduced markedly by only the 1.0 mgl^{-1} (1000 ppb) concentration of atrazine. A 50% reduction in the mean number of shoots was produced within approximately 16 days according to the regression analysis. Other concentrations of atrazine effected little change in the number of shoots during the test period.

In each of the morphometric data sets, it is significant to note that the control treatment resulted in an increase in mean height, mean number of leaves, and mean number of shoots over the course of the test period. Tests of the statistical significance of differences between control treatments and atrazine treatments are inconclusive, however, because of the highly variable nature of the morphometric data.

During the experiments reported here, the minimum water temperature averaged 22.2°C and the maximum water temperature averaged 27.3°C. Temperature usually fluctuated between these values daily.

DISCUSSION

The long-term dosing experiments reported here clearly demonstrated that atrazine at high concentrations (approximately 1 mgl^{-1} or 1000 ppb) can reduce the productivity of Zostera marina. The regression analysis utilized in this study, suggests major changes in morphology of Zostera may be produced by long-term exposure to atrazine concentrations as low as 0.06 mgl^{-1} (60 ppb). We believe the twenty one day EC_{50} are actually much higher than this value. A review of Figure 6.3 indicates that the trend established by the data points appears sigmoidal rather than linear. We have attempted more sophisticated analyses of the data, unfortunately, we do not have enough data points at high concentrations to allow a more rigorous determination of the twenty one day

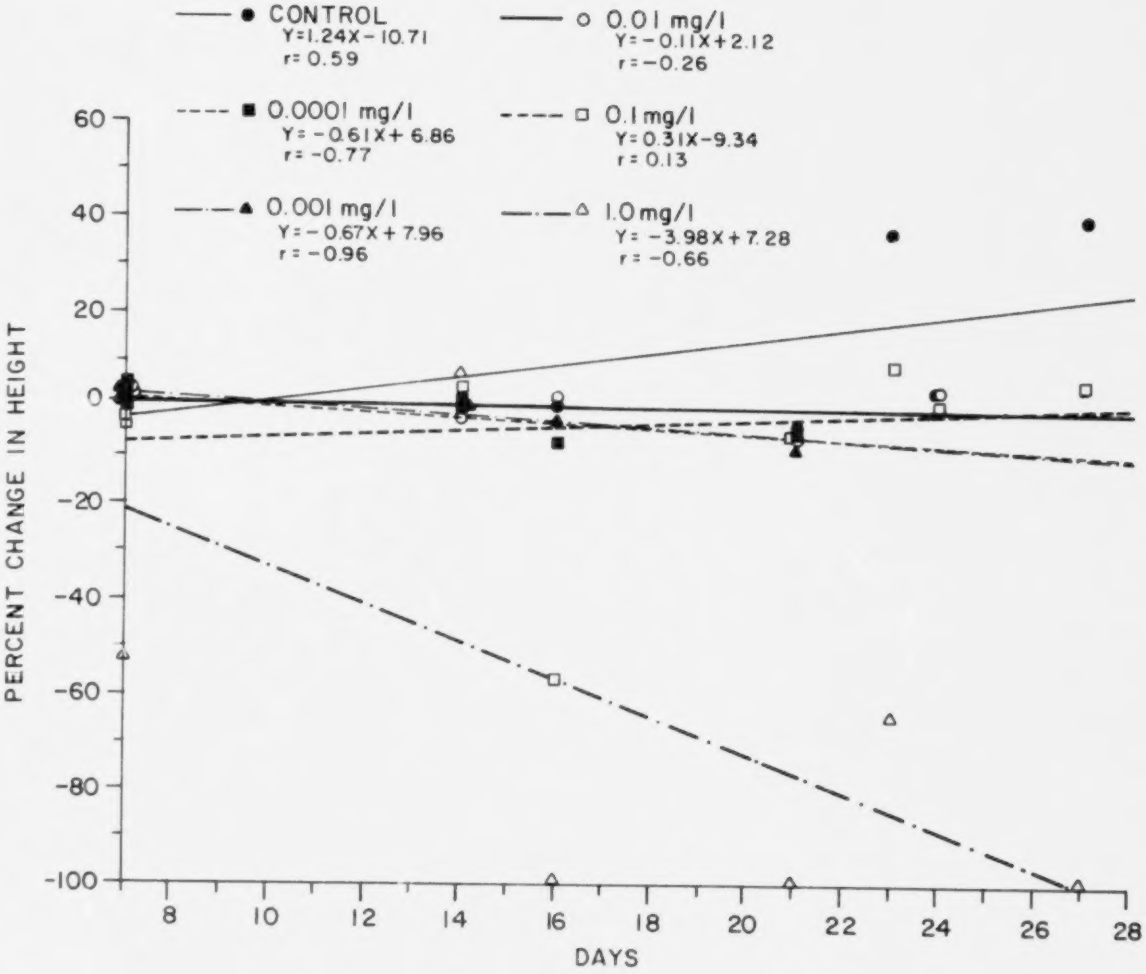

Figure 6.4. Graph of percent change in height of Zostera marina vs. time for six concentrations of atrazine (control, 0.0001 mgl^{-1}, 0.001 mgl^{-1}, 0.01 mgl^{-1}, 0.1 mgl^{-1}, and 1.0 mgl^{-1}).

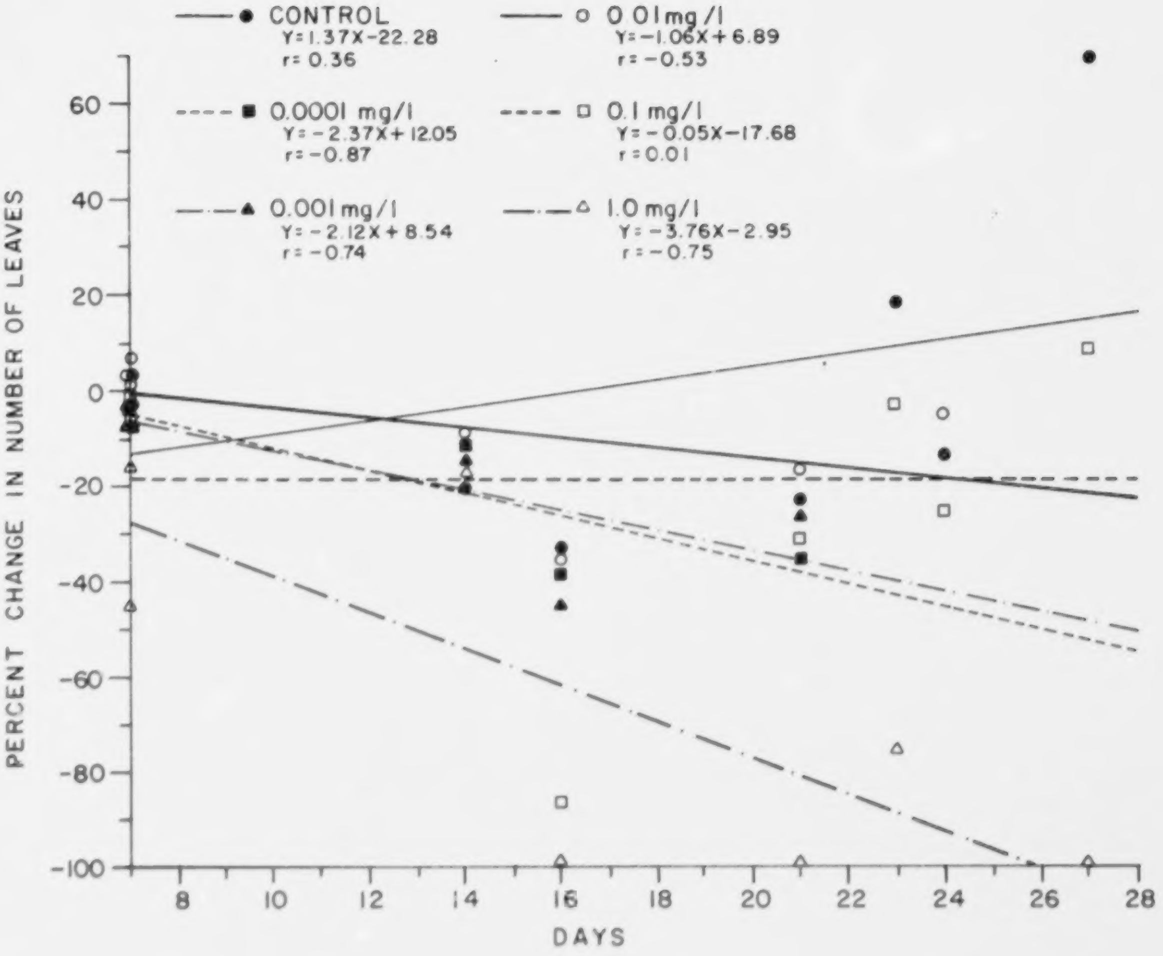

Figure 6.5. Graph of percent change in number of leaves of Zostera marina vs. time for six concentrations of atrazine (control, 0.0001 mgl^{-1}, 0.001 mgl^{-1}, 0.01 mgl^{-1}, 0.01 mgl^{-1}, and 1.0 mgl^{-1}).

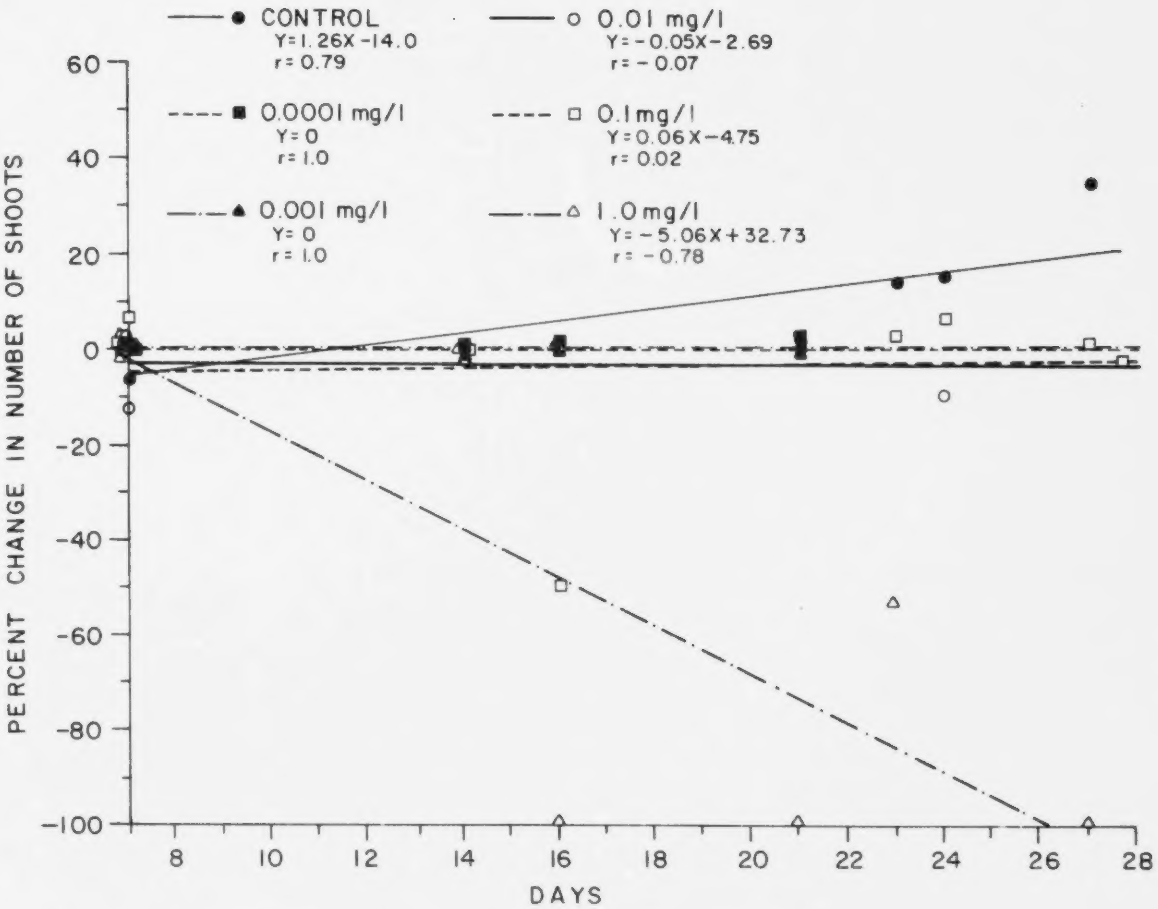

Figure 6.6. Graph of percent change in number of shoots of Zostera marina vs. time for six concentrations of atrazine (control, 0.0001 mgl^{-1}, 0.001 mgl^{-1}, 0.01 mgl^{-1}, 0.1 mgl^{-1} and 1.0 mgl^{-1})

LC_{50} or EC_{50}'s. The data points on Figure 6.3 suggest the EC_{50}'s for the morphometric parameter are all somewhere over 0.1 mgl^{-1} (100 ppb). The same suggestion is made by the data points used to establish the LC_{50} (Figure 6.2).

With either interpretation of the data several observations are significant. First, the effective concentrations of atrazine for production of a 50% decrease in selected morphological parameters are much higher concentrations than either of our survey programs found in Bay waters. Additionally, these experiments exposed _Zostera_ to atrazine concentrations for longer periods of time than we believe occur in natural conditions. Finally, our experiments do not indicate whether the effects of atrazine exposure persist after _Zostera_ plants are returned to unstressed conditions.

It is obvious from these studies that efforts to define atrazine EC_{50}'s and LC_{50}'s for _Zostera marina_ will need to focus on concentrations between 0.1 mgl^{-1} (100 ppb) and 1.0 mgl^{-1} (1000 ppb). These studies were not designed that way because our interest was principally in the very low concentrations found by the survey work to be typical of lower Bay waters. It should also be obvious from these studies that gross morphology is not sufficiently responsive to detect effects at the levels of replication we have employed. Either much larger numbers of plants will be required or an alternative, more sensitive test parameter must be employed.

TABLE D6.1. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #1 DATE: 5-13-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.1mg/1 atrazine						1.0mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	15.2	4	1	19.7	8	2	18.4	7	1	17.6	6	1	13.8	6	1	17.2	6	1
	21.5	5	1	12.3	2	1	18.8	5	1	23.4	9	2	17.6	5	1	19.8	3	1
	14.8	5	1	17.5	5	1	22.2	5	1	21.0	4	1	24.3	12	2	17.0	4	1
	18.2	6	2	14.0	5	1	19.1	10	2	15.8	7	1	20.9	10	1	17.3	6	1
	14.3	6	1	17.4	9	2	23.4	6	1	14.9	5	1	21.3	5	1	13.2	4	1
	18.3	4	1	18.1	6	1	18.7	5	1	12.7	4	1	22.8	6	1	20.6	6	2
	14.7	4	1	14.8	5	1	11.6	4	1	27.7	5	1	14.8	6	1	14.6	5	1
	18.7	5	1	13.2	6	1	17.2	5	1	18.0	5	1	24.5	11	2	13.5	5	1
	22.0	4	1	11.4	4	1	16.7	10	2	19.1	4	1	12.3	5	1	15.4	5	1
	12.3	4	1	19.7	8	2	8.6	2	1	18.4	5	1	18.7	7	1	16.1	6	1
	23.0	4	1	20.2	4	1	20.5	8	2	22.0	10	2	19.8	6	1	20.8	11	2
	17.3	4	1	21.6	11	2	17.4	5	2	18.8	6	1	18.8	9	1	19.6	5	1
	10.1	3	1	20.1	4	1	21.3	5	1	19.5	6	1	18.5	5	1	20.7	5	1
	21.9	9	2	20.1	7	1	23.2	2	2	20.1	6	1	17.7	4	1	16.8	9	2
	19.4	10	2	28.5	6	1	9.8	3	1	21.0	9	2	19.1	4	1	17.2	5	1
$\bar{x}$	17.5	5.1	1.2	17.9	6.0	1.3	17.8	5.5	1.3	19.3	6.1	1.2	19.0	6.7	1.1	17.3	5.7	1.2
σ	3.8	2.0	0.4	4.4	2.3	0.5	4.6	2.5	0.5	3.6	1.9	0.4	3.5	2.6	0.4	2.5	2.0	0.4
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.2. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #1 DATE: 6-9-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

0.0mg/1 atrazine						0.1mg/1 atrazine						1.0mg/1 atrazine					
Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
21.4	11	2	30.6	11	2	11.4	3	1	20.2	9	1	-	-	-	-	-	-
27.2	9	1	25.2	5	1	24.1	9	2	22.0	5	1	-	-	-	-	-	-
19.7	8	1	27.3	11	1	23.4	4	1	22.7	5	1	-	-	-	-	-	-
22.9	8	1	25.7	11	2	17.3	5	1	17.8	8	1	-	-	-	-	-	-
18.7	7	1	24.6	23	4	21.4	8	2	18.4	14	2	-	-	-	-	-	-
26.1	9	2	26.1	11	2	-	-	-	19.5	4	1	-	-	-	-	-	-
18.2	6	1	23.4	9	2	19.2	7	2	20.7	4	1	-	-	-	-	-	-
27.6	7	1	24.3	8	1	19.3	4	1	19.1	5	1	-	-	-	-	-	-
28.5	7	2	20.6	5	1	11.5	1	1	26.8	5	1	-	-	-	-	-	-
17.3	5	1	24.3	10	2	19.8	7	1	15.5	3	1	-	-	-	-	-	-
29.8	7	1	28.7	9	2	24.1	8	1	14.7	3	1	-	-	-	-	-	-
24.7	6	1	33.4	19	3	19.6	11	3	16.7	8	1	-	-	-	-	-	-
13.2	4	1	23.7	9	2	21.7	4	1	20.8	5	1	-	-	-	-	-	-
31.6	15	2	21.3	10	2	19.0	5	1	23.4	9	2	-	-	-	-	-	-
25.0	15	3	30.2	9	2	18.2	9	2	18.9	6	1	-	-	-	-	-	-
x̄23.5	8.3	1.4	26.0	10.7	1.9	19.3	6.1	1.4	19.8	6.5	1.1						
σ5.3	3.2	0.6	3.5	4.7	0.8	3.9	2.8	0.7	3.2	3.4	0.4						
n15	15	15	15	15	15	14	14	14	15	15	15						

(-)=plants died

TABLE D6.3. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #2 DATE: 5-27-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.1mg/1 atrazine						1.0mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	13.7	7	1	19.3	6	1	19.4	5	1	13.4	4	1	22.5	12	2	19.1	7	1
	12.9	5	1	19.9	10	2	23.1	17	3	26.3	9	1	14.0	6	1	15.2	4	1
	16.1	6	1	17.1	5	1	21.7	10	1	21.6	9	1	18.2	2	1	15.7	6	1
	14.1	5	1	16.2	5	1	19.8	12	2	14.3	5	1	7.4	3	1	17.7	5	1
	23.8	13	2	18.5	6	1	17.7	6	1	8.5	4	1	22.3	10	1	25.8	9	1
	17.7	4	1	18.7	13	1	15.7	4	1	21.4	5	1	17.0	9	1	16.2	7	1
	21.3	11	2	17.6	8	1	13.3	6	1	19.2	6	1	15.9	6	1	19.4	7	1
	19.5	6	1	17.2	6	1	19.2	6	1	5.6	3	1	13.6	5	1	20.3	11	2
	20.2	4	1	11.5	1	1	23.1	9	2	14.7	6	1	12.2	4	1	15.3	4	1
	18.9	11	2	22.0	8	2	24.1	5	1	18.7	6	1	15.1	4	1	21.2	4	1
	23.2	14	2	18.2	5	1	22.5	11	2	20.7	5	1	16.3	5	1	20.1	5	1
	15.6	7	1	17.5	9	1	22.6	7	1	22.5	4	1	17.2	4	1	26.4	6	1
	17.0	6	1	10.9	4	1	18.2	5	1	16.2	6	1	15.1	6	1	21.1	6	1
	17.2	5	1	14.6	5	1	18.3	7	1	19.2	10	1	23.2	10	2	18.6	6	1
	24.4	11	2	19.3	6	1	16.2	6	1	20.6	15	3	18.7	8	1	17.2	5	1
$\bar{x}$	18.4	7.7	1.3	17.2	6.5	1.1	19.7	7.7	1.3	17.5	6.5	1.1	16.6	6.3	1.1	19.3	6.1	1.1
σ	3.7	3.4	0.5	3.0	2.8	0.4	3.2	3.5	0.6	5.5	3.1	0.5	4.2	2.9	0.4	3.4	1.9	0.3
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.4. MORPHOMETRIC MEASUREMENTS OF *ZOSTERA MARINA* EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #2 DATE: 6-19-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.1mg/1 atrazine						1.0mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	24.0	6	1	23.2	5	1	19.3	6	1	28.6	9	1	-	-	-	21.3	3	1
	22.0	8	1	18.7	4	1	18.8	7	1	9.1	3	1	-	-	-	10.5	3	1
	29.8	12	2	17.3	3	1	18.1	5	1	18.8	4	1	-	-	-	19.0	3	1
	26.7	20	3	21.4	12	2	28.7	8	2	6.2	3	1	-	-	-	9.4	2	1
	27.6	6	1	-	-	-	25.1	5	1	24.3	9	1	-	-	-	12.2	4	1
	26.8	12	2	18.2	7	2	24.3	7	1	16.2	4	1	-	-	-	7.8	3	1
	26.7	8	2	29.3	8	2	25.3	9	2	22.0	7	1	-	-	-	-	-	-
	26.0	17	3	26.7	8	1	15.1	5	1	29.3	8	2	-	-	-	-	-	-
	26.5	4	1	25.1	13	2	15.2	3	1	18.6	7	1	-	-	-	-	-	-
	23.4	10	2	21.2	8	1	22.0	6	1	18.7	4	1	-	-	-	-	-	-
	35.0	6	1	23.8	6	1	20.7	5	1	27.9	6	1	-	-	-	-	-	-
	22.0	9	1	23.9	7	1	21.2	12	2	23.8	16	3	-	-	-	-	-	-
	22.0	8	1	24.7	10	1	24.0	11	1	19.3	11	1	-	-	-	-	-	-
	22.7	5	1	28.4	8	1	25.7	14	3	22.2	4	1	-	-	-	-	-	-
	25.6	5	1	21.8	3	1	20.0	5	1	15.7	6	1	-	-	-	-	-	-
$\bar{x}$	25.8	9.5	1.5	23.1	7.3	1.3	21.6	7.1	1.3	19.1	6.7	1.2				13.7	3.0	1.0
σ	3.5	4.7	0.7	3.7	3.0	0.5	4.0	3.1	0.6	5.9	3.5	0.6				5.1	0.6	0
n	15	15	15	14	14	14	15	15	15	15	15	15				6	6	6

(-)=plants died

TABLE D6.5. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #3 DATE: 6-23-80

(H = height of tallest shoot in cm; L - number of leaves per pot; S = number of shoots per pot)

0.00mg/1 atrazine						0.01mg/1 atrazine						0.10mg/1 atrazine					
Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
22.3	6	1	21.3	12	2	13.7	4	1	12.1	3	1	23.9	9	1	15.7	6	1
15.8	5	1	20.6	9	1	18.3	5	1	20.6	9	1	16.2	6	1	14.7	5	1
14.8	3	1	17.3	4	1	12.1	4	1	16.5	4	1	15.0	5	1	25.7	13	2
23.1	6	1	20.2	4	1	23.3	8	2	22.2	13	2	23.1	5	1	25.0	5	1
14.2	5	1	17.3	4	1	20.1	4	1	14.8	7	1	16.2	5	1	18.4	6	1
22.2	5	1	11.9	4	1	20.3	7	1	14.7	4	1	20.5	5	1	26.0	9	1
15.3	4	1	17.8	4	1	21.1	18	2	15.6	5	1	6.8	2	1	20.7	5	1
12.4	3	1	16.7	4	1	17.2	5	1	16.7	4	1	16.3	6	1	26.9	12	1
14.1	4	1	25.3	9	1	10.6	2	1	20.6	5	1	20.1	6	1	18.3	4	1
18.3	5	1	17.7	5	1	22.3	8	2	23.6	10	2	17.2	5	1	14.3	6	1
15.8	6	2	24.3	5	1	16.3	5	1	23.6	6	1	16.3	5	1	12.0	5	1
19.0	6	1	20.2	11	2	19.0	4	1	22.5	6	1	15.3	7	2	17.3	5	1
23.0	11	1	13.7	5	1	22.1	7	1	24.6	7	1	20.7	11	2	17.6	5	1
24.5	4	1	19.3	9	1	20.7	4	1	18.8	14	3	18.7	6	1	17.0	5	1
18.7	4	1	15.6	4	1	20.0	5	1	26.4	13	3	14.8	3	1	15.3	5	1
x̄18.2	5.1	1.1	18.6	6.2	1.1	18.8	6.00	1.2	19.6	7.3	1.4	17.4	5.7	1.1	19.0	6.4	1.1
σ4.0	1.9	0.3	3.6	2.9	0.4	3.4	3.7	0.4	4.3	3.6	0.7	4.1	2.2	0.4	4.8	2.7	0.3
n15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.6. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #3 DATE: 6-30-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.00mg/1 atrazine						0.01mg/1 atrazine						0.10mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	23.1	13	1	16.4	4	1	22.5	7	1	26.2	14	1	15.8	4	1	16.3	4	1
	24.2	4	1	19.4	9	1	20.3	5	1	20.6	5	1	18.9	5	1	18.4	5	1
	20.2	5	1	13.6	4	1	18.9	4	1	23.0	7	1	16.0	14	3	12.0	5	1
	18.9	6	1	25.8	8	1	16.8	5	1	25.1	6	1	15.5	8	2	17.3	6	1
	20.2	5	2	18.7	4	1	21.9	4	1	19.2	11	1	20.1	7	2	11.6	4	1
	19.7	6	1	24.8	5	1	11.2	2	1	23.6	6	1	16.4	5	1	17.1	5	1
	14.1	4	1	20.7	10	1	22.4	9	2	14.8	6	1	17.7	4	1	20.9	6	1
	22.8	6	1	17.7	4	1	21.2	19	2	16.9	5	1	21.1	4	1	18.6	5	1
	16.6	4	1	12.2	4	1	17.3	5	1	15.0	5	1	6.8	3	1	26.6	8	1
	12.6	4	1	18.1	4	1	23.1	8	2	17.0	4	1	14.6	7	1	17.0	12	1
	15.6	5	1	17.7	4	1	20.1	4	1	16.9	5	1	16.6	4	1	18.3	5	1
	22.3	6	1	26.7	11	1	14.2	4	1	22.2	14	2	16.7	6	1	25.3	5	1
	16.2	5	1	20.8	10	1	18.6	5	1	25.2	11	1	16.8	6	1	26.1	10	2
	15.7	3	1	21.0	5	1	16.7	4	1	11.3	4	1	22.6	6	1	14.1	4	1
	23.7	5	1	17.7	6	1	21.2	8	1	21.4	9	1	24.2	10	1	15.4	5	1
$\bar{x}$	19.1	5.4	1.1	19.0	6.1	1.0	19.1	6.2	1.2	19.9	7.5	1.1	17.3	6.2	1.3	18.7	5.9	1.1
σ	3.7	2.3	0.3	3.6	2.7	0	3.4	4.0	0.4	4.5	3.5	0.3	4.0	2.8	0.6	4.4	2.3	0.3
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.7. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #3 DATE: 7-17-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

0.00mg/1 atrazine						0.01mg/1 atrazine						0.10mg/1 atrazine					
Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
24.3	5	1	16.9	4	1	16.9	4	1	23.7	8	1	24.2	7	1	15.2	4	1
17.2	3	1	21.6	4	1	18.8	5	1	12.1	3	1	22.0	4	1	14.3	3	1
17.3	4	1	20.8	8	2	13.7	5	1	27.9	13	1	16.5	4	1	25.5	7	2
16.9	4	1	20.7	12	3	20.7	9	1	22.7	1	2	16.8	4	1	25.1	4	1
12.7	4	1	17.5	4	1	19.7	3	1	17.2	5	1	17.4	3	1	18.7	4	1
16.0	4	1	16.6	3	1	22.3	8	2	17.1	4	1	15.0	5	1	26.7	13	1
21.4	6	2	26.7	4	1	17.8	4	1	18.1	5	1	5.6	1	1	26.8	6	2
21.2	4	2	11.8	3	1	22.6	19	2	16.5	5	1	21.1	3	1	18.0	3	1
19.9	5	1	17.2	4	1	22.2	8	1	15.3	4	1	18.3	4	1	20.1	3	1
13.8	3	1	21.6	10	3	9.4	2	1	26.7	5	1	15.7	4	1	16.9	3	1
21.7	4	1	19.2	4	1	22.1	4	1	20.1	10	2	19.1	5	1	12.6	4	1
21.2	9	1	25.7	7	2	16.9	5	1	26.2	5	1	14.8	3	1	17.0	3	1
22.3	4	1	13.7	3	1	14.6	4	1	25.1	6	1	20.2	4	1	18.9	3	1
22.6	4	1	18.4	7	1	21.1	5	1	20.5	4	1	19.7	12	3	16.0	4	1
18.7	4	1	16.6	3	1	22.4	7	1	25.9	10	2	15.3	5	2	17.2	4	1
$\bar{x}$19.2	4.5	1.1	19.0	5.3	1.4	18.8	6.1	1.1	21.0	6.5	1.2	17.5	4.5	1.2	19.3	4.5	1.1
σ3.4	1.5	0.4	4.0	2.8	0.7	3.9	4.1	0.4	4.9	3.1	0.4	4.3	2.5	0.6	4.6	2.6	0.4
n15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.8. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #4 DATE: 9-9-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.0001mg/1 atrazine						0.001mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	23.5	3	1	20.3	2	1	25.1	4	1	31.5	4	1	18.3	4	1	25.5	5	1
	14.5	4	1	12.3	4	1	14.7	5	1	14.3	4	1	25.4	5	1	17.8	4	1
	12.6	3	1	21.7	5	1	20.6	6	1	25.2	4	1	17.7	3	1	24.2	4	1
	25.4	4	1	21.9	3	1	32.3	3	1	23.5	4	1	23.9	3	1	24.9	5	1
	15.7	3	1	27.3	4	1	24.1	5	1	29.4	4	1	23.8	4	1	18.9	5	1
	28.1	4	1	15.0	3	1	25.8	4	1	19.2	4	1	24.0	5	1	21.7	4	1
	10.6	3	1	17.7	3	1	21.9	5	1	13.3	4	1	20.6	4	1	20.4	4	1
	16.2	4	1	18.3	4	1	15.5	4	1	16.5	4	1	25.0	5	1	17.3	3	1
	24.8	3	1	25.3	4	1	16.5	4	1	21.8	2	1	12.0	4	1	22.2	3	1
	21.3	4	1	13.9	4	1	27.5	4	1	23.2	4	1	12.8	4	1	19.7	3	1
	26.2	4	1	19.0	4	1	20.6	4	1	25.5	4	1	14.1	4	1	13.1	4	1
	22.2	4	1	16.5	4	1	19.9	5	1	24.0	4	1	20.2	4	1	30.6	4	1
	20.0	4	1	22.6	3	1	16.1	3	1	17.1	3	1	18.5	3	1	14.2	3	1
	14.4	4	1	30.1	4	1	18.1	4	1	30.3	3	1	17.7	4	1	14.8	5	1
	25.2	4	1	17.9	4	1	19.3	5	1	17.0	5	1	18.3	4	1	17.9	4	1
$\bar{x}$	20.0	3.7	1.0	19.7	3.7	1.0	21.2	4.3	1.0	22.1	3.8	1.0	19.5	4.0	1.0	20.2	4.0	1.0
σ	5.6	0.5	0	5.2	0.7	0	5.0	0.8	0	5.8	0.7	0	4.4	0.7	0	4.8	0.8	0
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

(-)=plants died

TABLE D6.9. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #4 DATE: 9-9-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.01mg/1 atrazine						0.1mg/1 atrazine						1.0mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	15.4	4	1	22.5	3	1	25.2	4	1	27.9	5	1	17.8	3	1	17.5	4	1
	23.2	4	1	22.0	4	1	17.2	4	1	17.1	3	1	11.0	3	1	23.0	4	1
	19.7	5	1	21.1	3	1	24.1	3	1	24.2	4	1	27.7	3	1	17.8	4	1
	27.1	4	1	21.4	4	1	27.8	4	1	20.2	4	1	20.0	4	1	25.0	3	1
	25.3	4	1	27.3	3	1	29.0	4	1	28.6	5	1	26.8	5	1	15.3	4	1
	27.3	4	1	21.7	4	1	18.3	4	1	14.8	4	1	17.4	4	1	24.4	5	1
	19.3	5	1	22.0	3	1	24.1	4	1	17.4	4	1	9.2	3	1	17.4	3	1
	25.2	5	1	24.6	4	1	18.5	4	1	15.9	5	1	18.4	4	1	12.2	4	1
	16.3	3	1	22.8	3	1	11.2	3	1	18.2	5	1	27.1	4	1	22.5	4	1
	17.0	5	1	10.1	4	1	32.4	4	1	22.0	3	1	23.2	5	1	25.0	4	1
	17.2	4	1	23.1	4	1	20.4	3	1	24.5	4	1	22.3	4	1	12.7	3	1
	19.6	4	1	12.5	4	1	15.9	4	1	16.3	3	1	11.1	4	1	19.7	4	1
	11.5	3	1	26.9	4	1	14.0	3	1	16.7	5	1	22.2	4	1	16.6	4	1
	13.6	3	1	18.6	2	1	12.0	5	1	17.9	5	1	18.2	3	1	20.1	3	1
	24.5	5	1	22.9	4	1	23.6	4	1	12.0	3	1	17.4	3	1	19.0	4	1
$\bar{x}$	20.2	4.1	1.0	21.3	3.5	1.0	20.9	3.8	1.0	19.6	4.1	1.0	19.3	3.7	1.0	19.2	3.8	1.0
σ	5.0	0.7	0	4.6	0.6	0	6.4	0.6	0	4.9	0.8	0	5.8	0.7	0	4.2	0.6	0
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.10. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #4 DATE: 9-13-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.0001mg/1 atrazine						0.001mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	24.8	3	1	23.0	3	1	18.9	4	1	24.7	3	1	18.7	4	1	14.6	4	1
	24.5	4	1	13.9	5	1	20.2	4	1	25.6	3	1	12.7	3	1	13.2	4	1
	19.9	3	1	17.5	3	1	19.2	3	1	22.9	5	1	21.3	4	1	25.2	5	1
	22.2	4	1	30.6	4	1	34.5	4	1	18.0	3	1	14.7	3	1	19.2	4	1
	15.9	3	1	16.6	4	1	27.8	4	1	24.6	4	1	24.0	7	1	14.7	3	1
	25.6	4	1	13.2	4	1	18.2	4	1	17.2	4	1	27.0	4	1	19.3	3	1
	13.3	4	1	20.6	2	1	21.3	4	1	31.7	3	1	23.9	5	1	20.0	5	1
	22.4	5	1	21.7	4	1	25.5	5	1	29.2	4	1	20.0	4	1	30.3	3	1
	10.2	3	1	25.2	4	1	16.0	4	1	20.3	4	1	18.0	3	1	24.6	4	1
	26.7	4	1	23.3	4	1	25.6	4	1	21.3	2	1	24.0	3	1	22.5	4	1
	27.3	4	1	18.2	3	1	24.2	5	1	19.4	4	1	17.7	4	1	24.3	2	1
	16.8	4	1	16.9	4	1	17.6	3	1	15.5	4	1	24.3	5	1	18.4	4	1
	23.2	4	1	20.6	5	1	17.4	4	1	25.0	5	1	25.3	4	1	20.7	4	1
	14.8	4	1	27.7	4	1	23.1	5	1	15.9	4	1	19.2	4	1	20.3	3	1
	13.7	3	1	17.9	4	1	27.2	3	1	31.2	5	1	12.4	4	1	19.2	4	1
$\bar{x}$	20.1	3.7	1.0	20.5	3.8	1.0	22.5	4.0	1.0	22.8	3.8	1.0	20.2	4.1	1.1	20.4	3.7	1.0
σ	5.5	0.6	0	4.9	0.8	0	5.1	0.7	0	5.3	0.9	0	4.6	1.0	0	4.5	0.8	0
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.11. MORPHOMETRIC MEASUREMENTS OF *ZOSTERA MARINA* EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #4   DATE: 9-13-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.01mg/1 atrazine						0.1mg/1 atrazine						1.0mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	17.4	4	1	12.9	4	1	17.4	3	1	16.7	3	1	5.2	2	1	13.1	2	1
	17.7	3	1	29.0	4	1	12.7	3	1	18.1	4	1	12.0	2	1	3.1	1	1
	18.2	3	1	18.6	2	1	10.6	2	1	17.5	3	1	6.2	3	1	20.7	2	1
	27.3	5	1	25.2	4	1	33.2	4	1	17.5	4	1	5.9	2	1	1.5	1	1
	13.7	3	1	23.3	4	1	23.3	4	1	-	-	-	2.7	2	1	25.5	3	1
	25.7	5	1	9.7	3	1	15.1	2	1	12.1	2	1	17.2	2	1	14.0	3	1
	19.8	4	1	23.0	4	1	14.6	3	1	24.6	4	1	24.8	4	1	12.5	2	1
	11.9	3	1	23.6	4	1	20.4	3	1	22.6	3	1	2.7	2	1	1.6	2	1
	20.2	5	1	29.5	4	1	29.4	4	1	20.8	4	1	16.5	3	1	19.0	4	1
	26.5	4	1	25.1	4	1	25.0	5	1	26.5	4	1	1.5	2	1	2.3	1	1
	24.6	4	1	18.5	4	1	22.2	3	1	24.2	4	1	2.5	2	1	2.2	1	1
	20.1	4	1	21.2	4	1	10.7	1	1	14.2	4	1	3.2	3	1	3.3	1	1
	17.4	4	1	20.7	4	1	20.7	4	1	14.7	3	1	10.2	1	1	3.2	1	1
	27.6	4	1	24.4	4	1	18.2	5	1	28.7	2	1	21.7	3	1	4.0	1	1
	15.7	5	1	22.1	3	1	25.6	4	1	17.2	3	1	3.1	1	1	18.5	3	1
$\bar{x}$	20.3	4.0	1.0	21.8	3.7	1.0	19.9	3.3	1.0	19.7	3.4	1.0	9.0	2.3	1.0	9.6	1.9	1.0
σ	5.0	0.8	0	5.3	0.6	0	6.7	1.1	0	5.0	0.7	0	7.7	0.8	0	8.4	1.0	0
n	15	15	15	15	15	15	15	15	15	14	14	14	15	15	15	15	15	15

(-)=plants died

TABLE D6.12. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #4 DATE: 9-25-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.0001mg/1 atrazine						0.001mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	23.7	1	1	15.7	3	1	-	-	-	24.1	2	1	23.5	3	1	12.2	1	1
	8.2	3	1	16.7	2	1	-	-	-	17.3	1	1	20.7	3	1	19.4	1	1
	11.2	1	1	18.0	2	1	-	-	-	18.2	3	1	22.6	3	1	19.4	3	1
	22.6	3	1	20.3	2	1	-	-	-	16.1	2	1	14.3	2	1	30.5	2	1
	25.7	2	1	22.2	3	1	29.2	2	1	22.2	3	1	26.2	4	1	12.8	2	1
	14.3	3	1	30.0	1	1	24.6	3	1	29.0	1	1	16.8	3	1	10.2	1	1
	17.1	3	1	18.7	3	1	22.3	5	1	21.2	1	1	24.3	3	1	-	-	-
	20.9	3	1	19.9	4	1	13.4	2	1	11.6	1	1	24.3	3	1	-	-	-
	-	-	-	25.5	3		21.9	4	1	16.8	3	1	20.1	2	1	-	-	-
	-	-	-	32.7	2		17.2	3	1	17.5	3	1	20.0	1	1	-	-	-
	-	-	-	-	-	-	17.2	3	1	26.9	3	1	-	-	-	-	-	-
	-	-	-	-	-	-	18.2	2	1	17.0	1	1	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	20.8	3	1	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	9.3	2	1	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\bar{x}$	18.0	2.4	1.0	22.0	2.5	1.0	20.4	3.0	1.0	19.1	2.1	1.0	21.3	2.7	1.0	17.4	1.7	1.0
σ	6.3	0.9	0	5.7	0.9	0	4.9	1.1	0	5.4	0.9	0	3.7	0.8	0	7.5	0.8	0
n	8	8	8	10	10	10	8	8	8	14	14	14	10	10	10	6	6	6

(-)=plants died

TABLE D6.13. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #4 DATE: 9-25-80

(H = height of tallest shoot in cm; L = number of leaver per pot; S = number of shoots per pot)

0.01mg/1 atrazine						0.1mg/1 atrazine						1.0mg/1 atrazine					
Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
28.2	4	1	19.2	1	1	17.2	1	1	-	-	-	-	-	-	-	-	-
18.1	4	1	22.5	2	1	19.3	1	1	-	-	-	-	-	-	-	-	-
21.2	3	1	25.8	2	1	-	-	-	-	-	-	-	-	-	-	-	-
27.1	3	1	24.7	3	1	-	-	-	-	-	-	-	-	-	-	-	-
14.3	2	1	10.5	2	1	-	-	-	-	-	-	-	-	-	-	-	-
25.7	4	1	19.1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
20.2	3	1	25.1	3	1	-	-	-	-	-	-	-	-	-	-	-	-
20.3	2	1	23.0	3	1	-	-	-	-	-	-	-	-	-	-	-	-
16.7	1	1	20.0	2	1	-	-	-	-	-	-	-	-	-	-	-	-
24.7	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16.0	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\bar{x}$21.4	2.9	1.0	21.1	2.1	1.0	18.3	1.0	1.0									
σ4.7	0.9	0	4.7	0.8	0	1.5	0	-									
n11	11	11	9	9	9	2	2	2									

(-)=plants died

TABLE D6.14. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #5 DATE: 9-19-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.0001mg/1 atrazine						0.001mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	17.																	
	17.1	4	1	27.2	5	1	23.2	4	1	20.8	3	1	12.9	3	1	20.6	4	1
	15.8	2	1	24.1	3	1	16.2	3	1	28.8	4	1	12.6	3	1	18.2	4	1
	21.2	5	1	18.2	4	1	15.3	4	1	11.2	2	1	23.4	3	1	30.5	4	1
	10.8	2	1	21.0	4	1	14.3	4	1	15.7	5	1	20.5	4	1	24.9	4	1
	19.4	5	1	15.9	3	1	14.1	4	1	17.3	4	1	22.6	4	1	17.5	3	1
	12.9	4	1	23.1	5	1	18.3	4	1	22.1	4	1	26.4	3	1	16.7	3	1
	22.1	5	1	16.0	4	1	18.7	3	1	15.2	4	1	27.2	5	1	26.1	5	1
	13.6	3	1	23.7	4	1	23.9	4	1	20.1	4	1	19.7	3	1	19.9	4	1
	21.2	4	1	28.8	4	1	27.6	7	1	20.1	4	1	39.2	4	1	15.7	1	1
	13.7	4	1	15.2	3	1	19.3	4	1	23.8	4	1	12.8	4	1	21.3	3	1
	12.2	4	1	25.8	4	1	16.3	3	1	28.7	5	1	22.1	4	1	23.6	4	1
	22.7	4	1	18.8	5	1	18.3	3	1	27.5	4	1	16.8	5	1	18.8	3	1
	18.9	3	1	18.2	5	1	27.1	4	1	26.4	4	1	7.9	2	1	27.2	5	1
	18.8	5	1	14.8	4	1	24.5	4	1	17.4	4	1	20.8	3	1	26.4	3	1
	17.7	3	1	23.4	3	1	18.1	4	1	10.8	4	1	15.0	4	1	23.2	5	1
$\bar{x}$	17.2	3.8	1.0	21.0	4.0	1.0	19.7	3.9	1.0	20.4	4.0	1.0	20.0	3.6	1.0	22.0	3.7	1.0
σ	4.0	1.0	0	4.6	0.8	0	4.5	1.0	0	5.9	0.7	0	7.7	0.8	0	4.4	1.1	0
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.15. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #5 DATE: 9-19-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

0.01mg/l atrazine						0.1mg/l atrazine						1.0mg/l atrazine					
Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
34.6	4	1	20.9	5	1	19.7	4	1	17.2	3	1	22.3	4	1	23.5	4	1
29.6	4	1	7.0	3	1	19.2	5	1	16.5	3	1	24.7	4	1	18.1	2	1
15.7	3	1	27.3	6	1	19.0	4	1	21.6	2	1	17.7	3	1	25.2	4	1
11.1	3	1	21.8	5	1	19.6	5	1	22.0	2	1	12.5	4	1	18.7	4	1
22.2	4	1	16.6	4	1	18.8	4	1	9.9	3	1	19.3	3	1	24.9	5	1
24.3	4	1	17.4	3	1	16.6	3	1	23.8	4	1	21.7	5	1	13.5	3	1
10.1	3	1	30.2	4	1	26.7	4	1	12.0	3	1	13.8	4	1	12.3	3	1
29.3	4	1	27.2	5	1	18.6	4	1	15.2	4	1	9.4	4	1	21.6	4	1
21.9	4	1	24.5	3	1	20.1	3	1	18.1	4	1	13.0	4	1	16.6	4	1
12.3	3	1	24.7	4	1	22.6	7	1	19.7	3	1	19.2	5	1	21.7	4	1
26.4	4	1	21.3	4	1	22.8	4	1	19.3	4	1	27.0	4	1	20.3	4	1
13.1	4	1	22.4	3	1	21.9	3	1	13.3	3	1	14.1	3	1	28.9	5	1
19.4	3	1	12.8	3	1	23.3	4	1	22.1	3	1	18.7	4	1	17.2	2	1
22.7	4	1	17.4	3	1	22.1	5	1	20.6	4	1	19.6	4	1	10.1	3	1
23.7	2	1	22.0	5	1	18.2	4	1	12.7	4	1	26.4	6	1	25.1	4	1
$\bar{x}$21.1	3.5	1.0	20.9	4.0	1.0	20.6	4.2	1	17.6	3.3	1.0	18.6	4.1	1.0	19.9	3.7	1.0
σ7.4	0.6	0	6.0	1.0	0	2.6	1.0	0	4.2	0.7	0	5.3	0.8	0	5.3	0.9	0
n15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.16. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #5 DATE: 9-27-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

0.0mg/l atrazine						0.0001mg/l atrazine						0.001mg/l atrazine					
Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
13.9	1	1	20.2	2	1	23.5	2	1	24.0	3	1	31.7	3	1	26.5	3	1
22.5	1	1	16.1	3	1	13.2	2	1	22.0	3	1	21.4	1	1	24.1	4	1
20.1	1	1	18.1	3	1	16.2	2	1	30.1	5	1	11.4	1	1	16.1	1	1
18.5	3	1	24.3	2	1	25.7	3	1	28.1	3	1	19.2	1	1	4.8	1	1
15.7	2	1	27.2	3	1	18.9	3	1	17.1	3	1	12.7	3	1	19.3	4	1
14.4	2	1	24.0	2	1	28.0	4	1	16.7	3	1	17.1	4	1	15.5	3	1
18.0	2	1	9.3	2	1	19.2	4	1	24.1	4	1	20.1	4	1	14.7	3	1
-	-	-	34.0	4	1	14.3	2	1	26.7	4	1	15.4	4	1	4.3	1	1
-	-	-	18.5	3	1	18.7	3	1	17.2	3	1	26.2	2	1	26.6	3	1
-	-	-	17.8	2	1	25.2	3	1	19.7	1	1	19.1	1	1	-	-	-
-	-	-	-	-	-	18.2	3	1	3.0	1	1	13.2	3	1	-	-	-
-	-	-	-	-	-	19.2	4	1	12.2	4	1	17.7	3	1	-	-	-
-	-	-	-	-	-	16.3	4	1	20.7	3	1	-	-	-	-	-	-
-	-	-	-	-	-	23.2	3	1	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	14.0	3	1	-	-	-	-	-	-	-	-	-
$\bar{x}$17.6	1.7	1.0	21.0	2.6	1.0	19.6	3.0	1.0	20.1	3.1	1.0	18.8	2.5	1.0	18.0	2.6	1.0
σ3.1	0.8	0	6.8	0.7	0	4.6	0.8	0	7.2	1.1	0	5.8	1.2	0	8.8	1.2	0
n7	7	7	10	10	10	15	15	15	13	13	13	12	12	12	9	9	9

(-)=plants died

TABLE D6.17. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #5 DATE: 5-27-80

0.01mg/1 atrazine						0.1mg/1 atrazine						1.0mg/1 atrazine					
Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
18.5	2	1	22.0	1	1	10.3	1	1	20.0	3	1	26.1	2	1	5.1	1	1
22.5	3	1	25.0	2	1	23.1	2	1	12.2	2	1	19.1	2	1	6.0	1	1
29.2	4	1	27.5	2	1	17.7	2	1	13.5	1	1	11.0	1	1	18.1	1	1
14.0	3	1	26.0	2	1	16.8	1	1	22.2	2	1	-	-	-	-	-	-
23.1	1	1	18.7	2	1	9.7	1	1	18.2	4	1	-	-	-	-	-	-
29.5	1	1	8.5	1	1	17.8	2	1	8.2	1	1	-	-	-	-	-	-
10.5	1	1	12.2	1	1	6.5	1	1	22.7	1	1	-	-	-	-	-	-
-	-	-	-	-	-	18.5	1	1	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	20.1	3	1	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\bar{x}$21.0	2.1	1.0	20.0	1.6	1.0	15.6	1.6	1.0	16.7	2.0	1.0	18.7	1.7	1.0	9.7	1.0	1.0
σ7.2	1.2	0	7.3	0.5	0	5.5	0.7	0	5.5	1.2	0	7.6	0.6	0	7.3	0	0
n7	7	7	7	7	7	9	9	9	7	7	7	3	3	3	3	3	3

(-)=plants died

TABLE D6.18. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #5 DATE: 10-4-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.0001mg/1 atrazine						0.001mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	-	-	-	15.5	3	1	24.7	3	1	22.3	3	1	10.4	1	1	-	-	-
	-	-	-	27.5	2	1	23.5	2	1	13.7	2	1	15.4	2	1	-	-	-
	-	-	-	-	-	-	19.2	2	1	25.7	2	1	27.0	3	1	-	-	-
	-	-	-	-	-	-	17.5	3	1	23.8	3	1	-	-	-	-	-	-
	-	-	-	-	-	-	13.8	2	1	16.3	1	1	-	-	-	-	-	-
	-	-	-	-	-	-	25.9	2	1	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	21.3	2	1	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\bar{x}$				21.5	2.5	1.0	20.8	2.3	1.0	20.4	2.2	1.0	17.6	2.0	1.0			
σ				8.5	0.7	0	4.3	0.5	0	5.1	0.8	0	8.5	1.0	0			
n				2	2	2	7	7	7	5	5	5	3	3	3			

(-)=plants died

TABLE D6.19. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #5 DATE: 10-4-80

(H = height of tallest shoot in cm; L = number of leaver per pot; S = number of shoots per pot)

0.01mg/1 atrazine						0.1mg/1 atrazine						1.0mg/1 atrazine					
Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
30.7	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\bar{x}$ 30.7	1.0	1.0															
σ -	-	-															
n 1	1	1															

(-)=plants died

TABLE D6.20. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #5 DATE: 10-14-80

(H = height of tallest shoot in cm; L = number of leaver per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.0001mg/1 atrazine						0.001mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	-	-	-	-	-	-	25.4	3	1	-	-	-	20.1	1	1	-	-	-
	-	-	-	-	-	-	20.7	2	1	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	21.5	2	1	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\bar{x}$							22.5	2.3	1.0				20.1	1.0	1.0			
σ							2.51	0.6	0				-	-	-			
n							3	3	3				1	1	1			

(-)=plants died

Table 21.	0.01mg/1 atrazine ALL PLANTS DIED	0.1mg/1 atrazine ALL PLANTS DIED	1.0mg/1 atrazine ALL PLANTS DIED

TABLE D6.21. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #6 DATE: 10-5-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0 mg/1 atrazine						0.0001 mg/1 atrazine						0.001 mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	23.9	1	1	23.9	4	1	16.0	3	1	15.0	3	1	26.7	3	1	20.0	3	1
	25.7	3	1	26.7	3	1	23.7	3	1	31.6	5	1	21.6	3	1	18.9	3	1
	17.2	3	1	24.3	5	1	26.2	4	1	18.3	2	1	25.7	4	1	12.9	4	1
	17.1	3	1	20.0	3	1	20.1	3	1	23.9	4	1	20.1	5	1	18.2	4	1
	23.2	3	1	18.2	3	1	21.3	3	1	29.7	5	1	16.7	3	1	21.2	3	1
	26.5	4	1	33.2	5	1	33.0	5	1	30.5	3	1	18.7	2	1	23.1	2	1
	29.9	4	1	23.9	4	1	21.0	4	1	21.3	3	1	21.5	4	1	17.0	3	1
	21.2	3	1	23.0	2	1	18.2	3	1	21.3	3	1	25.9	4	1	21.7	4	1
	17.6	3	1	22.0	3	1	17.2	2	1	26.4	4	1	27.7	3	1	23.2	4	1
	21.2	3	1	27.7	3	1	16.2	3	1	19.3	2	2	25.2	2	1	27.0	4	1
	21.7	4	1	27.2	4	1	29.5	4	1	25.2	3	1	21.7	3	1	22.2	3	1
	22.9	4	1	23.6	3	1	28.6	4	1	15.5	3	1	24.6	4	1	19.0	4	1
	25.9	3	1	17.5	3	1	29.1	3	1	20.1	4	1	32.1	3	1	24.2	3	1
	30.7	4	1	20.0	2	1	14.2	2	1	19.0	3	1	18.8	4	1	20.7	2	1
	17.7	3	1	22.5	3	1	17.7	3	1	21.4	3	1	22.6	3	1	27.7	2	1
$\bar{x}$	22.8	3.3	1.0	23.5	3.3	1.0	22.1	3.3	1.0	22.6	3.3	1.0	23.3	3.3	1.0	21.1	3.2	1.0
σ	4.4	0.6	0	4.0	0.9	0	5.9	0.8	0	5.2	0.9	0	4.1	0.8	0	3.8	0.8	0
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.22. MORPHOMETRIC MEASUREMENTS OF *ZOSTERA MARINA* EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #6 DATE: 10-5-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

0.01 mg/1 atrazine						0.1 mg/1 atrazine						1.0 mg/1 atrazine					
Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
32.6	3	1	30.6	4	1	23.7	3	1	27.5	3	1	25.1	3	1	19.6	2	1
16.7	1	1	28.7	4	1	30.1	5	1	23.8	4	1	19.3	2	1	24.2	4	1
26.4	3	1	27.2	3	1	32.1	4	1	18.8	3	1	33.6	5	1	29.8	4	1
22.5	3	1	29.1	4	1	15.6	1	1	18.3	2	1	24.0	3	1	24.3	2	1
35.2	3	1	31.7	3	1	28.0	4	1	21.2	3	1	25.6	3	1	24.1	3	1
28.4	3	1	22.0	2	1	28.2	4	1	27.2	3	1	26.0	5	1	24.6	3	1
28.2	4	1	22.9	3	1	22.7	4	1	34.1	5	1	22.7	2	1	32.2	3	1
26.7	4	1	33.1	5	2	21.6	3	1	27.2	4	1	26.7	3	1	16.2	2	1
24.4	4	1	22.5	3	1	22.0	3	1	21.5	3	1	32.7	4	1	16.7	3	1
28.4	3	1	22.1	4	1	23.0	3	1	14.2	2	1	25.7	4	1	13.7	3	1
25.1	5	1	18.6	5	1	26.5	3	1	22.7	3	1	27.5	3	1	17.2	2	1
25.6	3	1	28.3	3	1	22.2	4	1	23.2	3	1	21.0	3	1	22.0	4	1
22.5	4	1	20.7	2	1	28.3	3	1	16.6	2	1	25.5	4	1	17.7	4	1
24.2	2	1	16.8	4	1	23.7	3	1	27.3	2	1	17.4	3	1	24.6	4	1
26.2	3	1	30.7	3	1	25.4	4	1	22.2	3	1	28.5	3	1	15.3	3	1
$\bar{x}$26.2	3.2	1.0	25.7	3.5	1.1	24.9	3.4	1.0	23.1	3.0	1.0	25.4	3.3	1.0	21.5	3.1	1.0
σ4.3	0.9	0	5.1	0.9	0.3	4.1	0.9	0	5.1	0.9	0	4.4	0.9	0	5.4	0.8	0
n15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.23. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #6 DATE: 10-12-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.0001mg/1 atrazine						0.001mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	31.5	1	1	22.7	4	1	29.1	3	1	20.5	3	1	31.7	4	1	26.1	3	1
	18.9	3	1	22.5	3	1	18.2	3	1	20.2	4	1	22.6	4	1	25.2	4	1
	27.0	4	1	17.2	2	1	27.7	5	1	18.3	1	1	27.4	3	1	21.3	3	1
	24.7	3	1	22.5	3	1	31.5	4	1	26.3	4	1	23.3	3	1	25.0	3	1
	17.7	2	1	22.0	2	1	16.3	3	1	15.1	2	1	21.6	3	1	20.6	2	1
	21.8	3	1	27.0	3	1	15.8	3	1	23.1	2	1	31.3	2	1	19.5	4	1
	31.0	4	1	25.1	3	1	17.2	1	1	19.9	3	1	19.2	3	1	23.2	5	1
	27.3	3	1	19.0	3	1	19.9	3	1	24.2	4	1	17.3	2	1	21.1	3	1
	20.3	4	1	23.6	2	1	22.5	4	1	21.2	3	1	20.1	3	1	21.7	3	1
	22.8	5	1	24.3	3	1	16.2	2	1	31.8	3	1	27.1	4	1	17.2	2	1
	23.1	3	1	33.4	4	1	19.8	3	1	21.5	3	1	27.7	3	1	23.1	2	1
	17.6	4	1	23.5	3	1	23.7	3	1	31.4	4	1	20.6	3	1	20.5	2	1
	27.1	4	1	26.2	3	1	23.2	3	1	32.7	5	1	25.7	4	1	20.6	3	1
	24.0	2	1	19.8	3	1	32.6	4	1	18.2	2	1	21.3	3	1	17.9	3	1
	16.9	4	1	25.9	4	1	22.1	4	1	14.2	2	1	22.0	3	1	12.4	3	1
$\bar{x}$	23.5	3.4	1.0	23.7	3.0	1.0	22.4	3.2	1.0	22.6	3.0	1.0	23.9	3.1	1.0	21.0	3.0	1.0
σ	4.7	0.8	0	3.8	0.7	0	5.6	0.9	0	5.8	1.7	0	4.4	0.6	0	3.5	0.9	0
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

TABLE D6.24. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #6 DATE: 10-12-80

(H = height of tallest shoot in cm; L = number of leaver per pot; S = number of shoots per pot)

	0.01mg/1 atrazine						0.1mg/1 atrazine						1.0mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	27.5	3	1	19.3	4	1	28.0	5	1	16.9	1	1	22.0	2	1	17.4	2	1
	23.1	3	1	21.3	4	1	23.2	3	1	22.2	3	1	24.6	2	1	21.3	3	1
	25.3	5	1	27.8	4	1	26.5	4	1	28.2	3	1	33.5	4	1	-	-	-
	25.1	4	1	22.2	3	1	22.4	4	1	15.0	1	1	-	-	-	13.4	4	1
	28.2	4	1	16.6	3	1	22.2	4	1	20.1	3	1	26.3	4	1	18.2	3	1
	22.6	3	1	22.5	3	1	22.2	3	1	24.0	2	1	25.7	2	1	25.4	3	1
	26.2	3	1	30.2	3	1	28.2	3	1	22.7	3	1	28.1	1	1	15.2	3	1
	35.1	3	1	22.0	3	1	20.2	1	1	27.8	3	1	-	-	-	25.1	3	1
	29.7	4	1	31.5	4	1	28.9	4	1	20.7	3	1	35.2	3	1	22.0	3	1
	28.3	4	1	32.7	3	1	22.6	3	1	33.7	4	1	25.3	2	1	30.5	3	1
	33.1	4	1	31.7	5	1	29.6	4	1	29.2	3	1	18.2	2	1	-	-	-
	22.3	3	1	32.9	5	1	22.8	4	1	21.7	3	1	-	-	-	24.7	2	1
	26.2	3	1	28.8	4	1	-	-	-	18.2	1	1	24.3	4	1	30.7	4	1
	16.8	1	1	26.8	2	1	32.0	4	1	17.9	2	1	24.3	2	1	-	-	1
$\bar{x}$	26.5	3.3	1.0	26.6	3.8	1.1	25.1	3.6	1.0	23.1	2.5	1.0	25.6	2.4	1.0	21.7	2.9	1.0
σ	4.5	0.9	0	5.5	1.2	0.3	3.7	0.9	0	5.3	0.9	0	5.0	1.1	0	5.8	0.7	0
n	15	15	15	15	15	15	14	14	14	15	15	15	12	12	12	12	12	12

(-)=plants died

TABLE D6.25. MORPHOMETRIC MEASUREMENTS OF *ZOSTERA MARINA* EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #6 DATE: 10-19-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0mg/1 atrazine						0.0001mg/1 atrazine						0.001mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	20.7	3	1	18.7	2	1	17.9	2	1	14.7	2	1	28.1	3	1	20.7	3	1
	19.2	2	1	27.3	2	1	16.3	3	1	20.3	3	1	22.7	3	1	18.2	3	1
	22.5	3	1	16.7	3	1	28.7	5	1	25.4	3	1	29.3	3	1	21.3	2	1
	27.6	3	1	27.7	3	1	24.1	3	1	20.7	3	1	19.4	1	1	20.3	4	1
	23.9	3	1	24.2	2	1	31.2	2	1	18.2	2	1	22.0	3	1	23.2	2	1
	27.5	2	1	24.5	3	1	19.3	4	1	31.7	4	1	19.7	3	1	13.2	3	1
	18.0	2	1	23.2	3	1	16.8	2	1	32.7	3	1	21.3	4	1	18.3	2	1
	23.4	2	1	25.2	2	1	20.1	3	1	31.4	4	1	19.7	2	1	21.7	2	1
	18.3	4	1	23.7	1	1	16.1	2	1	21.6	3	1	21.9	4	1	27.2	3	1
	23.2	3	1	18.7	2	1	23.5	3	1	27.4	3	1	26.7	3	1	21.2	2	1
	26.8	3	1	22.7	3	1	23.1	2	1	15.7	3	1	19.9	3	1	25.7	2	1
	31.7	4	1	21.2	3	1	21.3	4	1	20.7	2	1	21.0	4	1	20.7	2	1
	28.7	3	1	22.5	2	1	32.5	3	1	20.1	4	1	28.0	3	1	25.9	3	1
	15.4	3	1	-	-	-	21.3	3	1	23.7	2	1	-	-	-	23.5	3	1
	-	-	-	-	-	-	20.6	3	1	-	-	-	-	-	-	20.2	3	1
$\bar{x}$	23.4	2.9	1.0	22.8	2.4	1.0	22.2	2.9	1.0	23.2	2.9	1.0	23.1	3.0	1.0	21.4	2.6	1.0
σ	4.7	0.7	0	3.3	0.7	0	5.2	0.9	0	5.8	0.7	0	3.7	0.8	0	3.5	0.6	0
n	14	14	14	13	13	13	15	15	15	14	14	14	13	13	13	15	15	15

(-)=plants died

TABLE D6.26. MORPHOMETRIC MEASUREMENTS OF *ZOSTERA MARINA* EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #6 DATE: 10-19-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.01 mg/1 atrazine						0.1 mg/1 atrazine						1.0 mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	27.7	4	1	20.2	2	1	23.7	3	1	28.4	3	1	24.5	1	1	25.6	1	1
	27.8	3	1	24.6	3	1	29.1	2	1	21.5	3	1	-	-	-	-	-	-
	25.3	3	1	27.3	3	1	25.8	4	1	28.5	3	1	-	-	-	-	-	-
	23.6	4	1	22.4	3	1	22.2	4	1	16.7	1	1	-	-	-	-	-	-
	26.3	4	1	30.8	4	1	22.7	4	1	34.0	4	1	-	-	-	-	-	-
	23.5	3	1	33.5	4	1	28.2	3	1	21.0	2	1	-	-	-	-	-	-
	20.7	3	1	31.2	2	1	21.7	3	1	22.1	2	1	-	-	-	-	-	-
	25.7	2	1	34.0	4	1	22.6	3	1	27.5	3	1	-	-	-	-	-	-
	28.0	4	1	17.8	2	1	31.4	3	1	-	-	-	-	-	-	-	-	-
	33.4	3	1	27.7	3	1	21.2	3	1	-	-	-	-	-	-	-	-	-
	24.9	2	1	22.2	3	1	29.8	3	1	-	-	-	-	-	-	-	-	-
	32.0	3	1	23.2	2	1	21.7	2	1	-	-	-	-	-	-	-	-	-
	16.9	1	1	28.4	3	1	28.0	3	1	-	-	-	-	-	-	-	-	-
	24.2	4	1	15.4	3	1	-	-	-	-	-	-	-	-	-	-	-	-
	27.7	4	1	22.6	3	1	-	-	-	-	-	-	-	-	-	-	-	-
$\bar{x}$	25.9	3.1	1.0	25.4	2.9	1.0	25.2	3.1	1.0	25.0	2.6	1.0	24.5	1.0	1.0	25.6	1.0	1.0
σ	4.1	0.9	0	5.6	0.7	0	3.6	0.6	0	5.6	0.9	0	-	-	-	-	-	-
n	15	15	15	15	15	15	13	13	13	8	8	8	1	1	1	1	1	1

(-)=Plants died

TABLE D6.27. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #6 DATE: 10-26-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

	0.0 mg/1 atrazine						0.0001 mg/1 atrazine						0.001 mg/1 atrazine					
	Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
	21.2	3	1	24.2	2	1	17.5	2	1	27.1	3	1	21.7	3	1	24.6	3	1
	18.0	1	1	16.8	2	1	17.2	2	1	19.8	2	1	21.5	3	1	23.4	3	1
	25.8	2	1	22.1	2	1	17.8	1	1	21.2	2	1	26.5	2	1	16.9	2	1
	19.2	2	1	17.3	2	1	19.2	2	1	1912	3	1	17.6	2	1	20.2	2	1
	23.0	3	1	27.2	2	1	27.1	4	1	20.2	1	1	20.6	1	1	20.8	3	1
	23.0	3	1	15.7	1	1	18.3	2	1	24.0	3	1	28.0	2	1	19.2	3	1
	27.8	2	1	14.7	1	1	29.9	2	1	16.0	1	1	23.0	2	1	11.7	3	1
	14.5	2	1	25.7	2	1	20.5	3	1	14.3	1	1	16.3	2	1	17.8	2	1
	29.8	2	1	24.3	2	1	21.5	1	1	19.8	2	1	19.3	3	1	19.7	3	1
	32.0	2	1	22.6	2	1	13.7	2	1	30.5	2	1	26.2	3	1	15.7	2	1
	23.4	3	1	23.7	2	1	20.1	3	1	16.2	2	1	17.9	2	1	21.9	3	1
	18.7	2	1	-	-	-	32.9	2	1	28.7	3	1	18.8	2	1	20.3	1	1
	27.8	3	1	-	-	-	19.5	2	1	-	-	-	20.4	3	1	20.1	3	1
	23.0	2	1	-	-	-	21.2	3	1	-	-	-	-	-	-	25.8	2	1
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\bar{x}$	23.4	2.3	1.0	21.3	1.8	1.0	21.2	2.2	1.0	21.4	2.1	1.0	21.4	2.3	1.0	19.9	2.5	1.0
σ	4.9	0.6	0	4.4	0.4	0	5.3	0.8	0	5.2	0.8	0	3.7	0.6	0	3.7	0.7	0
n	14	14	14	11	11	11	14	14	14	12	12	12	13	13	13	14	14	14

(-)=Plants died

TABLE D6.28. MORPHOMETRIC MEASUREMENTS OF ZOSTERA MARINA EXPOSED TO ATRAZINE IN A FLOW THROUGH DOSING SYSTEM

EXPERIMENT #6 DATE: 10-26-80

(H = height of tallest shoot in cm; L = number of leaves per pot; S = number of shoots per pot)

0.01 mg/1 atrazine						0.1 mg/1 atrazine						1.0 mg/1 atrazine					
Tank A			Tank B			Tank A			Tank B			Tank A			Tank B		
H	L	S	H	L	S	H	L	S	H	L	S	H	L	S	H	L	S
24.2	2	1	22.4	2	1	28.2	2	1	19.2	2	1	-	-	-	-	-	-
31.3	2	1	20.2	2	1	21.5	3	1	25.7	2	1	-	-	-	-	-	-
23.5	2	1	32.8	3	1	29.4	3	1	33.3	2	1	-	-	-	-	-	-
22.9	3	1	25.8	2	1	20.3	2	1	21.7	2	1	-	-	-	-	-	-
15.9	1	1	23.2	3	1	18.9	3	1	26.2	3	1	-	-	-	-	-	-
30.1	3	1	27.4	4	1	15.2	1	1	24.9	3	1	-	-	-	-	-	-
18.8	3	1	18.7	3	1	21.5	1	1	-	-	-	-	-	-	-	-	-
24.0	3	1	29.2	4	1	27.2	1	1	-	-	-	-	-	-	-	-	-
22.4	3	1	26.4	3	1	18.5	3	1	-	-	-	-	-	-	-	-	-
23.5	2	1	29.5	5	2	26.4	2	1	-	-	-	-	-	-	-	-	-
26.8	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21.7	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26.5	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18.8	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
x̄23.6	2.5	1.0	25.6	3.1	1.1	22.7	2.1	1.0	25.2	2.3	1.0						
σ4.2	0.7	0	4.4	1.0	0.3	4.8	0.9	0	4.8	0.5	0						
n14	14	14	10	10	10	10	10	10	6	6	6						

(-)=Plants died

SECTION 7

ADENYLATE ENERGY CHARGE STUDIES

GENERAL INTRODUCTION

The adenylate energy charge (EC) was first defined by Atkinson and Walton (1967):

$$EC = \frac{(ATP) + 1/2\ (ADP)}{(ATP) + (ADP) + (AMP)}$$

This ratio was proposed as a fundamental metabolic control parameter. As such, EC represents the metabolic energy state of the cell. Broad applications of EC include the following:

1) disciplines, ranging from cellular biochemistry (Atkinson, 1977) to community ecology (Wiebe and Bancroft, 1975);

2) different cellular and organismic types, prokaryote vs. eukaryote, autotroph vs. heterotroph, and single vs. multicellular organisms (Chapman et al., 1971); and

3) a range of environments, including marine (Karl and Holm-Hansen, 1978), estuarine (Mendelssohn and McKee, 1981), and terrestrial systems (Ching and Kronstad, 1972).

Recent application of EC measurement to higher plants is extensive, primarily involving agriculturally important crop species (e.g. Raymond and Pradet, 1980; Saglio et al., 1980; Bonzon et al., 1981; Quebedeaux, 1981; Hampp et al., 1982). In contrast, adenylate literature on seagrasses (Knauer and Ayers, 1977) is extremely limited. Plants respond to environmental stress in numerous ways (Levitt, 1972; Cottenie and Camerlynck, 1979; Rabe and Krebb, 1979). Since the metabolic energy state of an organism is sensitive to environmental variation, both natural and anthropogenic, EC has been advanced as an index of sublethal stress (Ivanovici, 1980).

Zostera marina (eelgrass), a submerged marine angiosperm, functions as a food source, habitat, nutrient pump, and sediment stabilizer. The basic biology (Setchell, 1929; Burkholder and Doheny, 1968; Harrison and Mann, 1975; Orth et al., 1981) and ecological value (McRoy and Helfferich, 1977; Stevenson and Confer, 1978; Phillips and McRoy, 1980; Wetzel et al., 1981) of Z. marina are well documented.

Historically and more recently, the distribution and abundance of Z. marina have undergone large fluctuations in the Chesapeake Bay (Orth and Moore, 1981). The reduction of eelgrass beds has been attributed to disease

(Renn, 1934), temperature increase (Orth, 1976), herbicide input (Stevenson and Confer, 1978), cownose ray disturbance (Orth, 1975), and to a lesser extent, dredging and boating activities (Orth, 1976). A reliable method to assess the metabolic state of eelgrass is, therefore, essential. Application of energy charge measurement to *Z. marina* is a logical choice.

Objectives

1. A major objective of this study was development of a methodology to quantitatively measure adenine nucleotides and adenylate energy charge (EC) in *Zostera marina* (eelgrass). The remaining objectives incorporated these optimized techniques.

2. Adenylates and EC were compared among *Z. marina* tissues, including leaf, leaf sheath, root plus rhizome, and seed pod. Comparative measurements were made on eelgrass epiphytes, aboveground *Ruppia maritima* (widgeongrass), and aboveground *Spartina alterniflora* (saltmarsh cordgrass).

3. Monthly variation of adenylates and EC was assessed in above and below-ground *Z. marina* tissue over a one year period. Associated environmental and morphometric data were collected.

4. Adenylate and EC responses to two atrazine levels over 6 hours, and five atrazine levels over 21 days, were assessed in *Z. marina* leaf tissue. Hourly production rates were measured during the 6 hour experiment. Weekly morphometric changes and mortality were examined over the 21 day atrazine exposure period.

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METHOD DEVELOPMENT

Introduction

Adenine nucleotides, expressed as the adenylate energy charge (EC) ratio, regulate cellular energetics (Atkinson, 1977). Problems associated with methodology for the determination of *in situ* adenine nucleotide levels may limit the utility of the EC concept (Pradet and Raymond, 1978; Karl, 1980; Ivanovici, 1980). Methodology must be tailored to the specific chemical characteristics of a particular biological material in order to accurately determine *in situ* levels of intracellular adenine nucleotides. In addition, ease of operation and reproducibility are essential to any useful analytical technique.

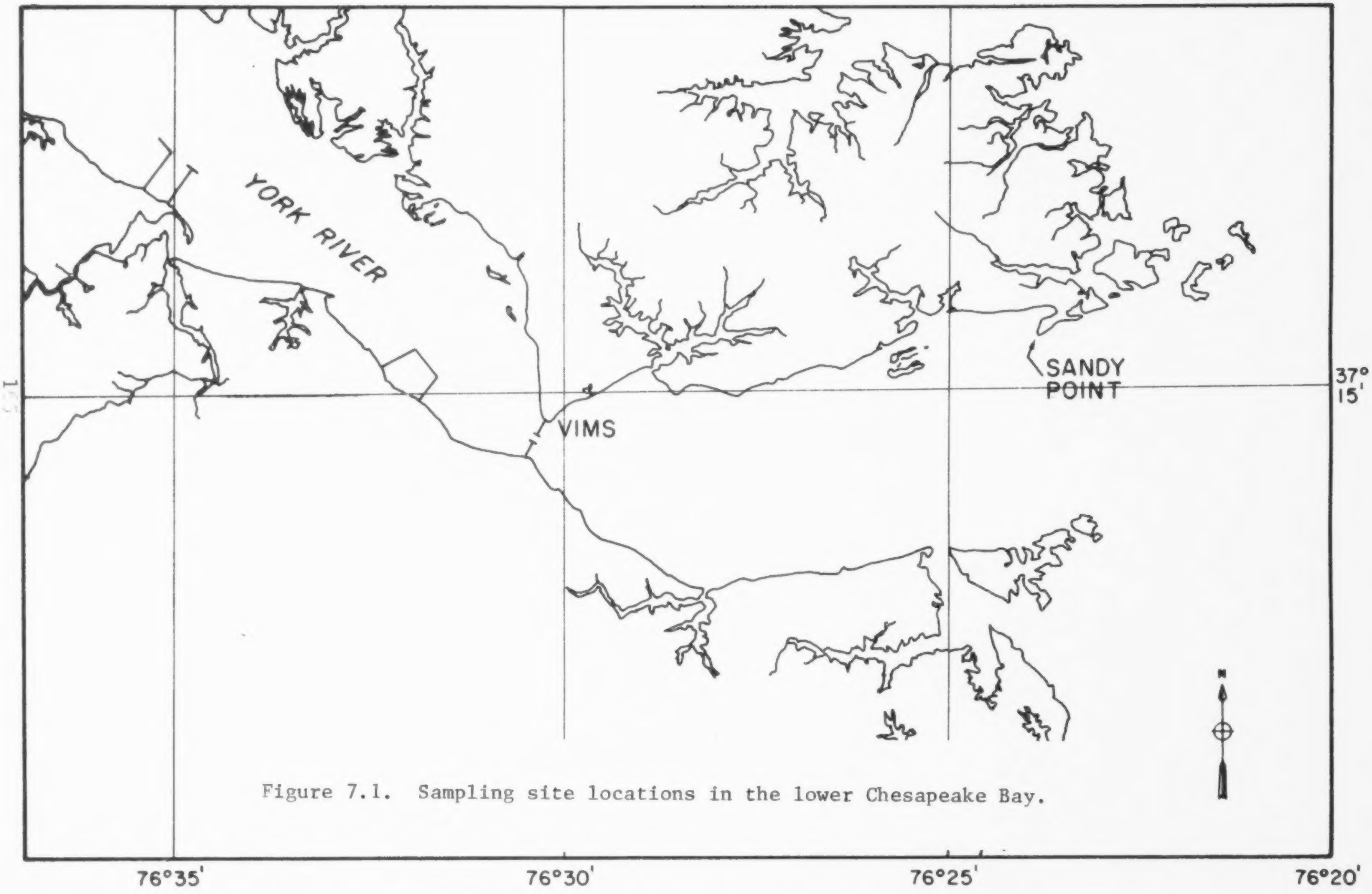
The most frequently employed methods for determination of adenine nucleotides involve enzymic conversion of adenosine monophosphate (AMP) and adenosine diphosphate (ADP) to equivalent amounts of adenosine triphosphate (ATP), followed by quantitative analysis of the ATP via the firefly bioluminescent reaction (Karl and Holm-Hansen, 1978). Determination of ATP by the firefly luciferase reaction, reviewed by Leach (1982), has been widely applied (DeLuca, 1978; DeLuca and McElroy, 1981). After reviewing the literature, Sofrova and Leblova (1970) concluded that the firefly reaction is the most rapid, sensitive, and specific method for ATP determination in plant tissue. Several studies which specifically address methodology for adenylate determination in higher plants utilize the firefly reaction (Pradet, 1967; Guinn and Eidenbock, 1972; DeGreef et al., 1979; Mendelssohn and McKee, 1981).

Employing the firefly assay, this study developed a methodology to optimize determination of adenine nucleotides in *Zostera marina* (eelgrass), a submerged marine angiosperm. *Z. marina* is an ecologically important macrophyte species (McRoy and Helfferich, 1977; Stevenson and Confer, 1978; Phillips and McRoy, 1980; Wetzel et. al., 1981; Orth et al., 1981), occurring in temperate and subarctic coastal and estuarine waters in the Northern Hemisphere (den Hartog, 1970). Major analytical procedures were evaluated, including sample collection and preparation, adenylate extraction, conversion of AMP and ADP to ATP, firefly lantern extract preparation, and photometry. Tissue composition and seasonal patterns of adenine nucleotides were also assessed in order to provide baseline information on natural adenylate variability in *Z. marina*.

Methods

Sampling Sites--

Zostera marina was collected at low tide from an extensive grassbed (37°15'40" N, 76°23'50" W) off Sandy Point at the mouth of the York River in the lower Chesapeake Bay estuary. This bed was close to the laboratory and accessible by land. Epiphytes and *Ruppia maritima* were also obtained from Sandy Point. *Spartina alterniflora* was collected from nearby Indian Field Creek (37°16'5" N, 76°33'30" W). Locations of these sites are shown in Figure 7.1.



YORK RIVER
VIMS
SANDY POINT
37° 15'
76°35'
76°30'
76°25'
76°20'

Figure 7.1. Sampling site locations in the lower Chesapeake Bay.

Adenine Nucleotide Methodology Experiments--

Assay principles--Adenylate assay reactions have been described by Pradet (1967), Holm-Hansen and Karl (1978), and DeLuca (1976). ATP is assayed with the firefly bioluminescent reaction (Figure 7.2). AMP and ADP are first converted enzymically to ATP (Figure 7.3), which is then analyzed by the firefly reaction. The equilibrium constant for the PK reaction is sufficiently large to convert most ADP, and consequently most AMP, to ATP (Adam, 1965).

Sample collection and preparation--Plants were uprooted with a shovel, swirled in river water to remove macro-algae and loose sediment, and stuffed in a 180 or 530 ml plastic bag (Whirl-Pak). Liquid nitrogen was poured into the bag (within 1 min of harvest) and the entire bag was submerged in liquid nitrogen contained in a 4 l polyethelene dewar flask (Nalgene) for return to the laboratory.

Liquid nitrogen was drained from the bag and the bag was then placed in a lyophilizer. The chamber was sealed and vacuum inititated, with condenser temperature allowed to reach -55°C before sample introduction. Chamber shelves, were not heated. Samples were lyophilized for 70-90 hrs.

After lyophilization, plant tissue was handled with forceps to prevent hydration. Brown aboveground tissue was discarded, since this material was considered dead at time of harvest. Leaves were scraped with a flat spatula which removes 70-90% of the epiphytes (Penhale, 1977).

For methodology experiments (excluding freeze delay), plants were pooled to provide a uniform substrate for experimental treatments. For tissue comparison and seasonal survey experiments, plants within a treatment (i.e. tissue type or monthly sample, respectively) were pooled in order to minimize within treatment variation. Leaf tissue was used for methodology experiments. Leaf, leaf sheath, root plus rhizome, and seed pod tissue were examined in the tissue comparison experiment. Aboveground (stem plus leaf) and belowground (root plus rhizome) parts were analyzed in the seasonal survey.

Tissues were ground in a cutting mill to pass a #40 (425μ) mesh screen. Scrapings (epiphytes) off lyophilized *Z. marina* leaves were ground by hand with mortar and pestle. Samples were either processed immediately or stored in a vacuum desiccator (Nalgene) in the dark for up to 5 days. Tissue preparation was adapted from the method of Mendelssohn and McKee (1981).

Extraction--Tissue was weighed into 20-80 mg aliquots and held in a desiccator. The extractant solution was 1 mM ethylenediaminetetraacetic acid (EDTA) + 5% (w/v) polyvinylpolypyrrolidone (PVPP) at pH 7.6. Four to eight ml of extractant are heated to 100°C in a 50 ml beaker on a hot plate (Corning). Tissue was added ($\leq$1% w/v), and the beaker swirled for 30 sec at 100°C. The extract was quantitatively transferred to a centrifuge tube. The beaker was rinsed with additional 1 mM EDTA which was poured into the centrifuge tube to bring the final volume up to 5 or 10 ml. These tubes were held on ice and centrifuged at 14000 g for 15 min at 4°C. The supernatant was decanted, held

$$E + LH_2 + ATP \xrightleftharpoons{Mg^{++}} E\text{-}LH_2AMP + PP_i$$

$$E\text{-}LH_2AMP + O_2 \longrightarrow E + \text{oxyluciferin} + AMP + CO_2 + h\nu$$

E: firefly luciferase (EC 1.13.12.7)

LH_2: luciferin

$E\text{-}LH_2AMP$: enzyme-bound luciferyl-adenylate

Figure 7.2. Firefly bioluminescent reaction.

ADP conversion

$$\text{ADP} + \text{PEP} \underset{K^{+},\ Mg^{++}}{\overset{\text{PK}}{\rightleftharpoons}} \text{ATP} + \text{pyruvate}$$

K = 2.89×10^3
pH 7.6
(Krimsky, 1959)

Coupled AMP conversion

$$\text{AMP} + \text{ATP} \underset{Mg^{++}}{\overset{\text{AK}}{\rightleftharpoons}} 2\ \text{ADP}$$

K = 1.2
(Atkinson, 1977)

$$\text{ADP} + \text{PEP} \underset{K^{+},\ Mg^{++}}{\overset{\text{PK}}{\rightleftharpoons}} \text{ATP} + \text{Pyruvate}$$

PEP:	phosphoenolpyruvate
PK:	pyruvate kinase (EC 2.7.1.40)
AK:	adenylate kinase (EC 2.7.4.3)

Figure 7.3. Enzymic conversion reactions.

on ice, and processed as soon as possible. Extraction mothodology was adapted from Mendelssohn and McKee (1981).

EDTA extractant solution was stored at 4°C in the dark and used for periods up to 1 month. A working aliquot of EDTA solution was vacuum-filtered through a 0.2 μ nitrocellulose membrane (Nalgene) for each day's analyses and discarded immediately after use. PVPP was added to the filtered EDTA solution approximately 30 min prior to extraction.

Conversion--AMP and ADP were enzymically converted to ATP. Three sets of reaction mixtures (13 x 100 mm disposable glass tubes) were prepared as follows:

Tube A (ATP Reagents):	400 μl blank (extractant), standard (ATP in extractant), or sample extract 400 μl reaction buffer (45 mM TRICINE, 18 mM $MgSO_4$, pH 7.6) 400 μl distilled water (DW)
Tube B (ADP + ATP Reagents):	400 μl blank, standard, or sample extract 400 μl reaction buffer 400 μl PK (30 μg), PEP (1.5 mM)
Tube C (AMP + ADP + ATP Reagents):	400 μl blank, standard, or sample extract 400 μl reaction buffer 400 μl PK, PEP, AK (30 μg).

These tubes were incubated (30°C, 30 min), heat deactivated (100°C, 2 min), and allowed to re-equilibrate (on ice, 20 min). Composition of conversion reaction mixtures with ATP standards appears in Table 7.1

Buffer was stored at 4°C in the dark and used for periods up to 2 weeks. Working aliquots of buffer and DW were filtered (0.2 μ) for each day's analyses and discarded immediately after use. Fresh solutions of [PEP + PK] and [PEP + PK + AK] were prepared in filtered DW in glass vials for each day's analyses, held on ice, and discarded immediately after use.

Firefly lantern extract preparation--One vial of lyophilized firefly lantern extract (FLE), commercially prepared from 50 mg dried lanterns, was hydrated with 25 ml filtered (0.2 μ) 45 mM TRICINE-18 mM $MgSO_4$ (pH 7.6) and aged (room temperature, 6-8 hrs) in order to degrade endogenous ATP. After aging, the insoluble residue was removed by centrifugation at 3000 RPM for 15 min. Whenever a large volume of FLE was required, several vials were pooled in order to eliminate variation between individual vials (Holm-Hansen and Karl, 1978).

Photometry--The photometer was allowed to warm up for at least 1 hr prior to assays. A sensitivity setting of 7.00 was utilized, since best instrument stability is achieved by using the lowest setting adequate for analysis (SAIT,

TABLE 7.1. COMPOSITION OF CONVERSION REACTION MIXTURES

Component	Units	Reaction Mixture		
		[ATP] Reagents	[ATP+ADP] Reagents	[ATP+ADP+AMP] Reagents
Na_2ATP	ng ml^{-1}	13-1333	13-1333	13-1333
TRICINE buffer	mM	15	15	15
$MgSO_4$	mM	6	6	6
Na_2EDTA	μM	333	333	333
PK	μg ml^{-1}	-	25	25
AK	μg ml^{-1}	-	-	25
Na_3PEP	μM	-	500	500
$(NH_4)_2SO_4$[1]	mM	-	15	30

[1] From PK and AK suspensions

1975). Dark current was nulled by adjusting the zero (4.80 - 4.90 at sensitivity 7.00), just before each standard set was run.

One hundred µl of solution from Tubes A, B, or C were pipetted (Eppendorf) into a 6 x 50 mm disposable glass tube. Fifty µl of FLE were pipetted (Eppendorf) into this tube, while simultaneously initiating the 10 sec delay mode of the photometer timing circuit with the footswitch. During this delay period, the tube was vortexed (Vortex-Genie) to ensure thorough mixing, inserted into the photometer, and the shutter opened. Counts were recorded for the following 10 sec integration period. A chart recorder was interfaced with the photometer to follow reaction kinetics in order to detect interferences or instrument malfunction.

For peak height measurements, 100 µl of solution from Tubes A, B, or C were pipetted into a 6 x 50 mm tube. The tube was placed inside the photometer, the shutter opened, and 50 µl of FLE injected with the electronic pipet system which simultaneously activates the photometer. Sensitivity settings from 7.00 - 10.00 were used. As a check on initial reagent mixing for peak height measurements, each tube was read, removed from the photometer, vortexed, and re-inserted into the photometer. If the recorder trace exhibited continuity, the reading was considered valid (Karl and Holm-Hansen, 1978). If not, the tube was discarded, and the process was repeated until a continuous trace (i.e. thorough initial mixing) was obtained.

Composition of firefly reaction mixtures with ATP standards appears in Table 7.2. In addition, pH values for reaction components and mixtures are presented in Table 7.3.

Standards and blanks--A primary standard was prepared with a weighed amount of ATP dissolved in filtered (0.2 µ), distilled, deionized water. This primary standard was divided into 1 ml aliquots and stored frozen (-20°C) in glass vials for a period up to 3 months. A fresh set of working standards was prepared in glass vials for each day's analyses. An aliquot of primary standard was thawed and serially diluted with filtered (0.2 µ) extractant solution (1 mM EDTA) to produce a set of standards which bracketed sample ATP levels. Working standards were held on ice and discarded immediately after use. Although Holm-Hansen and Karl (1978) reported no significant loss of these standard adenylates during an 8 hr period, a standard set was run at least every 2 hrs. Working standards and blanks were carried through enzymic conversion and incubation steps to parallel sample processing. This resulted in similar ionic composition and ATP reactivity, permitting more accurate adenylate quantification (Holm-Hansen and Karl, 1978). Standards and blanks were each read in duplicate per reaction Tube A, B, or C. In cases where a large discrepancy in duplicate readings occurred, a third reading was taken.

Another primary standard was prepared with weighed amounts of ATP, ADP, and AMP dissolved in filtered (0.2 µ), distilled, deionized water. The resultant standard, containing equal concentrations of ATP, ADP, and AMP, was used to calculate recovery and conversion efficiencies.

Data reduction--Net light output was computed by subtracting the appropriate blank value from each total light emission value. The log of net

TABLE 7.2. COMPOSITION OF FIREFLY REACTION MIXTURES

Component	Units	Reaction Mixture [ATP] Reagents	 [ATP+ADP] Reagents	 [ATP+ADP+AMP] Reagents
Na_2ATP	ng ml^{-1}	8-888	8-888	8-888
TRICINE buffer	mM	25	25	25
$MgSO_4$[1]	mM	11	11	11
Na_2EDTA	μM	222	222	222
PK	μg ml^{-1}	-	17	17
AK	μg ml^{-1}	-	-	17
Na_3PEP	μM	-	333	333
$(NH_4)_2SO_4$[2]	mM	-	10	20
FLE[3]	μg ml^{-1}	667	667	667
KH_2AsO_4[4]	mM	3	3	3

[1] 1 mM from FLE preparation
[2] From PK and AK suspensions
[3] Expressed as precursor firefly lanterns
[4] From FLE preparation

TABLE 7.3. REACTION COMPONENT AND MIXTURE PH VALUES

Label	Component or Mixture		pH[1]
R	Distilled water		7.45
S	75 μg ml^{-1} PK + 1.5 mM PEP		7.05
T	75 μg ml^{-1} PK + 1.5 mM PEP + 75 μg ml^{-1} AK		6.90
	1 mM EDTA		7.58
U	1 mM EDTA + 1 μg ml^{-1} ATP		7.66
V	45 mM TRICINE + 18 mM $MgSO_4$		7.62
W	[2]Tube A = 400 μl R + 400 μl U + 400 μl V	Conversion	7.55
X	[3]Tube B = 400 μl S + 400 μl U + 400 μl V	Reaction	7.55
Y	[4]Tube C = 400 μl T + 400 μl U + 400 μl V	Mixtures	7.53
Z	2 mg ml^{-1} FLE[5] + 45 mM TRICINE + 18 mM $MgSO_4$		7.43
	[2]Tube A = 100 μl W + 50 μl Z	Firefly	7.48
	[3]Tube B = 100 μl X + 50 μl Z	Reaction	7.49
	[4]Tube C = 100 μl Y + 50 μl Z	Mixtures	7.49

[1] pH meter calibrated with .05M (KH_2PO_4 - NaOH) buffer to pH 7.00 at 25°C
[2] [ATP] Reagents
[3] [ATP+ADP] Reagents
[4] [ATP+ADP+AMP] Reagents
[5] Expressed as precursor firefly lanterns

light emission (dependent variable) is regressed against the log of ATP concentration (independent variable) for three separate series of standards (Tubes A (ATP Reagents), B (ATP + ADP Reagents) and C (ATP + ADP + AMP Reagents)).

Each sample extract was similarly processed in reaction Tubes A, B, and C (duplicate reading per tube), and tube concentrations were calculated from corresponding standard regressions. Amounts of adenylates (ATP equivalents ml^{-1}) and EC were computed from tube concentrations as follows:

$$\begin{aligned} \text{ATP} &= \text{Tube A} \\ \text{ADP} &= \text{Tube B} - \text{Tube A} \\ \text{AMP} &= \text{Tube C} - \text{Tube B} \\ \text{AT} &= \text{Tube C} \\ \text{EC} &= \frac{\text{Tube A} + \text{Tube B}}{2(\text{Tube C})} \end{aligned}$$

An ATP equivalent is the amount of AMP, ADP, or AT, given as the weight of an equimolar amount of ATP (Pamatmat and Skjoldal, 1979). The formulation used for EC (Ball and Atkinson, 1975) reduces propagation of errors by using directly measured quantities. Since standards, blanks, and sample extracts all underwent identical dilution:

$$\frac{\mu\text{g ATP equivalent}}{\mu\text{g dry wt tissue}} = \frac{\mu\text{g ATP equivalent}}{\text{ml}} \times \frac{\text{ml extraction volume}}{\text{dry wt tissue}}$$

Recovery and conversion efficiencies--Efficiency of adenylate recovery after extraction was determined by assaying two aliquots: 1) sample with addition of known amounts of ATP, ADP, and AMP (internal standard) immediately before extraction, and 2) sample without internal standard addition. Recovery was calculated as follows (Mendelssohn and McKee, 1981):

% Recovery =

$$\frac{(AN_{\text{Tissue + Internal Standard}} - AN_{\text{Tissue}})\ \text{Determined by Assay}}{(AN_{\text{Internal Standard}})\ \text{Known Addition}} \times 100$$

where AN = ATP, ADP, or AMP.

Strehler (1968) offered two recommendations: 1) light output of sample plus added adenylate should not be more than 50% greater than the response of sample alone, and 2) maximum ATP concentration (sample plus internal standard) should be well below the K_m value for ATP with respect to luciferase (i.e. maintenance of linearity between light output and ATP concentration). Both of these recommendations were followed.

Efficiency of enzymic conversion (i.e. AMP and ADP to ATP) was evaluated by assaying a standard containing known of amounts of ATP, ADP, and AMP (Mendelssohn and McKee, 1981):

% Conversion =

$$\frac{(\mathrm{AN}_{\mathrm{Standard}})\ \text{Determined by Assay}}{(\mathrm{AN}_{\mathrm{Standard}})\ \text{Known Amount}} \times 100$$

where AN = ADP or AMP.

Reagents and equipment--The following reagents were obtained from Sigma Chemical Co.: firefly lantern extract (FLE-50), ATP (A 5394), ADP (A 6521), AMP (A 1877), GDP (G 6506), PEP (P 7002), PK (P 1506), AK (M 3003), PVPP (P 6755), TRIS-HCl (T 3253), HEPES (H 3375), and TRICINE (T 0377). Other chemicals used in this study were analytical reagent grade.

Adenylates were measured with an ATP photometer (Model 3000, SAI Technology Co.) and, in the case of peak height measurements, with the Enzyme Kinetics Kit electronic injection pipet (No. 020302, SAI Technology Co.). A chart recorder (Model 250/MM, Linear) was modified to accommodate an input voltage from 0.01-10 V.

Other equipment included a lyophilizer (Model 10-100, VirTis), mechanical analytical balance (Model H31, Mettler), electronic top-loading balance (Model PL 200, Mettler), drying oven (Model SW-17TA, Blue M Electric Co.), refrigerated centrifuge (Model PR-2, International Equipment Co.) with high capacity attachment, high speed angle centrifuge (Model SS-1,Sorvall), Thomas-Wiley intermediate mill (Model 3383-L10, Arthur H. Thomas Co.), water bath (Model MW-1110A-1, Blue M Electric Co.), vacuum pump (Millipore), and digital pH meter (Model 610, Fisher Scientific Co.), equipped with a glass-body combination electrode (No. 13-639-90, Fisher Scientific Co.). Disposable tubes, vials, filters, pipets, and pipet tips were routinely used. Reusable glassware was acid washed, rinsed 3 times with DW, and oven-dried to minimize contamination.

Adenine nucleotide methodology experiments--Differences between adenylates, subjected to various analytical treatments, were detected and located by the procedure diagrammed in Figure 7.4. Dependent variables are ATP, ADP, AMP, AT, and EC. Independent variables are treatment levels. The null hypothesis states no difference in adenylates between k treatments (i.e. $H_o : \mu_1 = \mu_2 = \ldots \mu_k$).

Standard curves, generated by three different photometer counting modes, were compared. Homogeneity of these linear regression slopes and intercepts was tested by analysis of covariance (ANCOVA). Data were log-transformed and satisfied the assumptions of homoscedasticity and normality. Pearson correlation coefficients for log-log regressions used in ANCOVA were calculated. Null hypotheses stated no difference in slopes ($H_o : \beta_1 = \beta_2 = \beta_3$) or intercepts($H_o : \alpha_1 = \alpha_2 = \alpha_3$) between regressions. Significant differences were located by the Student-Newman-Keuls multiple range test.

Tissue comparisons--Differences between adenylates in four tissue types were detected and located by the procedure diagrammed in Figure 7.4. Relationships among adenylates were evaluated by Spearman rank correlation.

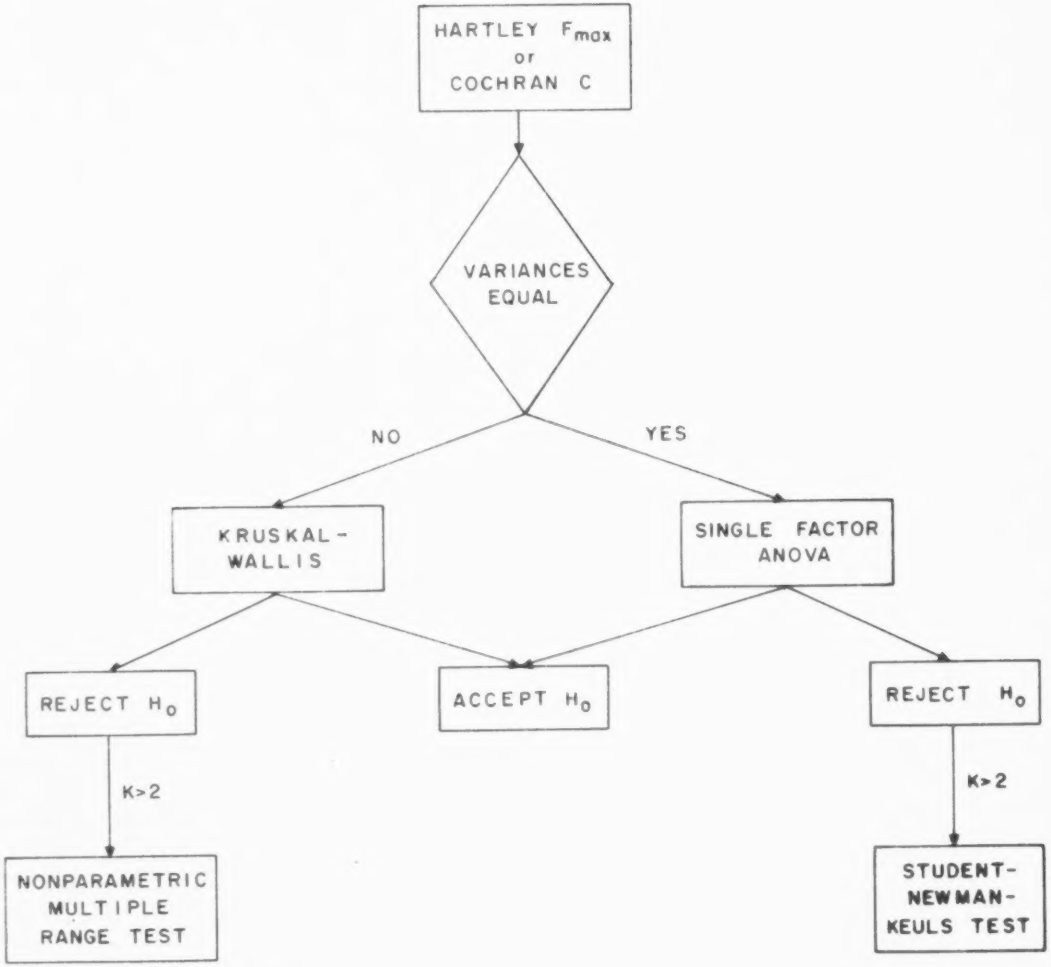

HARTLEY F_{max}
or
COCHRAN C
VARIANCES EQUAL
NO
YES
KRUSKAL-WALLIS
SINGLE FACTOR ANOVA
REJECT H_0
ACCEPT H_0
REJECT H_0
K>2
K>2
NONPARAMETRIC MULTIPLE RANGE TEST
STUDENT-NEWMAN-KEULS TEST

Figure 7.4. Detection and location of treatment differences.

Seasonal Survey--Differences between adenylates at monthly intervals were detected by the procedure diagrammed in Figure 7.4, although significant differences were not located. Relationships among adenylate, environmental, and morphometricd data were analyzed by Spearman rank correlation.

Environmental data include water temperature, salinity and pH. Water samples, collected in brown bottles (Nalgene), were returned to the laboratory for salinity (induction salinometer, Model RS 7B, Beckman) and pH measurements. Daylength and low tide time and height data were determined from tide tables (NOAA, 1979,1980).

At each harvest, one 0.03 m^2 plug of eelgrass, 10 cm deep, was collected with a plexiglass tube (0.10 m radius), placed in a coarse mesh bag, and washed free of sediment. This sample was returned to the laboratory and analyzed for total number of shoots, shoot lengths, and above and belowground biomass, according to Orth (1977).

Statistical Analysis--

The following procedures in the SPSS software package (Nie et al., 1975; Hull and Nie, 1981) were used: ONEWAY (single factor ANOVA, Hartley F max and Cochran C tests for homoscedasticity, Student-Newman-Keuls multiple range test), NPAR TESTS (Kruskal-Wallis single factor ANOVA by ranks and Kolmogorov-Smirnov one sample test for normality), and NONPAR CORR (Spearman rank correlation).

Other statistical procedures employed included analysis of covariance (test for homogeneity of linear regression slopes and intercepts) with an associated multiple range test (Zar, 1974), nonparametric multiple range testing by rank sums (Zar, 1974), linear regression, and Pearson correlation.

In standard curve regressions, ATP net count and concentration data were log-transformed. It was initially determined that log-transformed count data satisfy the assumptions of homoscedasticity and normality. Pearson correlation coeficients corresponding to these log-log regressions were calculated.

Results

Adenine Nucleotide Methodology Experiments--

Overview--Table 7.4 summarizes tested factors and their associated treatment levels, grouped under the appropriate analytical procedure. Standards and blanks, used to quantify samples and internal standard recovery and conversion, were processed in parallel with samples and internal standards for the following factors: extractant, all conversion factors, all FLE preparation factors, and photometer counting mode.

Sample collection and preparation--Eight harvest-freeze delay periods are compared in Table 7.5. The delay period represents the time interval between uprooting the plants and freezing in liquid nitrigen. ATP, AT, and EC generally increased as delay period lengthened. These trends are shown graphically in Figure 7.5. Associated regression statistics are presented in

TABLE 7.4. SUMMARY OF TESTED FACTORS

Procedure	Factor	Levels
Sample Collection and Preparation	harvest-freeze delay	.25, .5, 1, 2, 5, 10, 30, 60 min
	day vs night harvest with freeze delay	1200 hrs (30 sec, 10 min) 2400 hrs (30 sec, 10 min)
	tissue state	fresh-chopped (5 mm) vs frozen-lyophilized-ground (425 μ)
	epiphyte removal	scraped vs unscraped leaf
	desiccated-dark storage of frozen-lyophilized-ground tissue	0, 5, 20 days
Extraction	extractant	boiling 1 mM EDTA (pH 7.6), boiling 1 mM EDTA + 5% (w/v) PVPP (pH 7.6), boiling distilled water, 0-4°C .6 N H_2SO_4 + 1 mM EDTA (neutralized to pH 7.6-7.9 after extraction)
	extraction time	5, 30, 120 sec
	extracted tissue	individual vs pooled plants
	frozen extract storage	0, 5, 20 days

(continued)

TABLE 7.4. (continued)

Procedure	Factor	Levels
Conversion	buffer	15 mM TRICINE + 6 mM $MgSO_4$ (pH 7.6), 15 mM HEPES + 6 mM $MgSO_4$ (pH 7.6), 15 mM TRIS-HCl + 6 mM $MgSO_4$ (pH 7.6)
	enzyme cofactors	15 mM TRICINE (pH 7.6), 15 mM TRICINE + 6 mM $MgSO_4$ (pH 7.6), 15 mM TRICINE + 6 mM $MgSO_4$ + 7.5 mM K_2SO_4 (pH 7.6), 15 mM TRICINE + 6 mM $MgSO_4$ + 7.5 mM K_2SO_4 (pH 8.1)
	heat deactivation	heated, not heated
FLE Preparation	reconstituent	distilled water, 45 mM TRICINE + 18 mM $MgSO_4$ (pH 7.6), 45 mM TRICINE + 18 mM $MgSO_4$ (pH 8.1)
	aging time and temperature	6 hrs (4, 25°C), 24 hrs (4, 25°C)
	GDP addition	0, 6.85 $\mu g\ ml^{-1}$
Photometry	counting mode	peak height, 10 sec delay + 10 sec integral, 10 sec delay + 30 sec integral
	standard volume/FLE volume (peak height mode)	$\frac{20,\ 50,\ 100\ \mu l\ \text{standard}}{15,\ 25,\ 50,\ 100,\ 200\ \mu l\ \text{FLE}}$

TABLE 7.5. EFFECT OF TIME DELAY, BETWEEN HARVEST AND FREEZING, ON ADENINE NUCLEOTIDES (μg ATP equiv g^{-1} dry wt) and EC (n=2)

Variable	Time Delay (min)							
	.25	.5	1	2	5	10	30	60
ATP	138^{a}[1] ± 1[2]	134^{a} ± 4	157^{b} ± 2	140^{a} ± 1	216^{c} ± 6	196^{d} ± 1	184^{e} ± 3	240^{f} ± 3
ADP	91^{a} ± 1	91^{a} ± 3	103^{a} ± 1	98^{a} ± 4	68^{a} ± 13	91^{a} ± 5	102^{a} ± 1	106^{a} ± 1
AMP	78^{a} ± 4	97^{b} ± 3	84^{a} ± 1	73^{ac} ± 1	53^{d} ± 5	61^{cd} ± 4	82^{a} ± 5	63^{cd} ± 1
AT	306^{a} ± 2	322^{ab} ± 11	344^{bc} ± 3	311^{ab} ± 3	337^{abc} ± 15	347^{bc} ± 9	368^{c} ± 4	408^{d} ± 5
EC	$.60^{a}$ ± .01	$.56^{b}$ ± <.01	$.61^{a}$ ± <.01	$.61^{a}$ ± <.01	$.75^{c}$ ± .01	$.70^{d}$ ± .01	$.64^{e}$ ± .01	$.72^{f}$ ± <.01

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

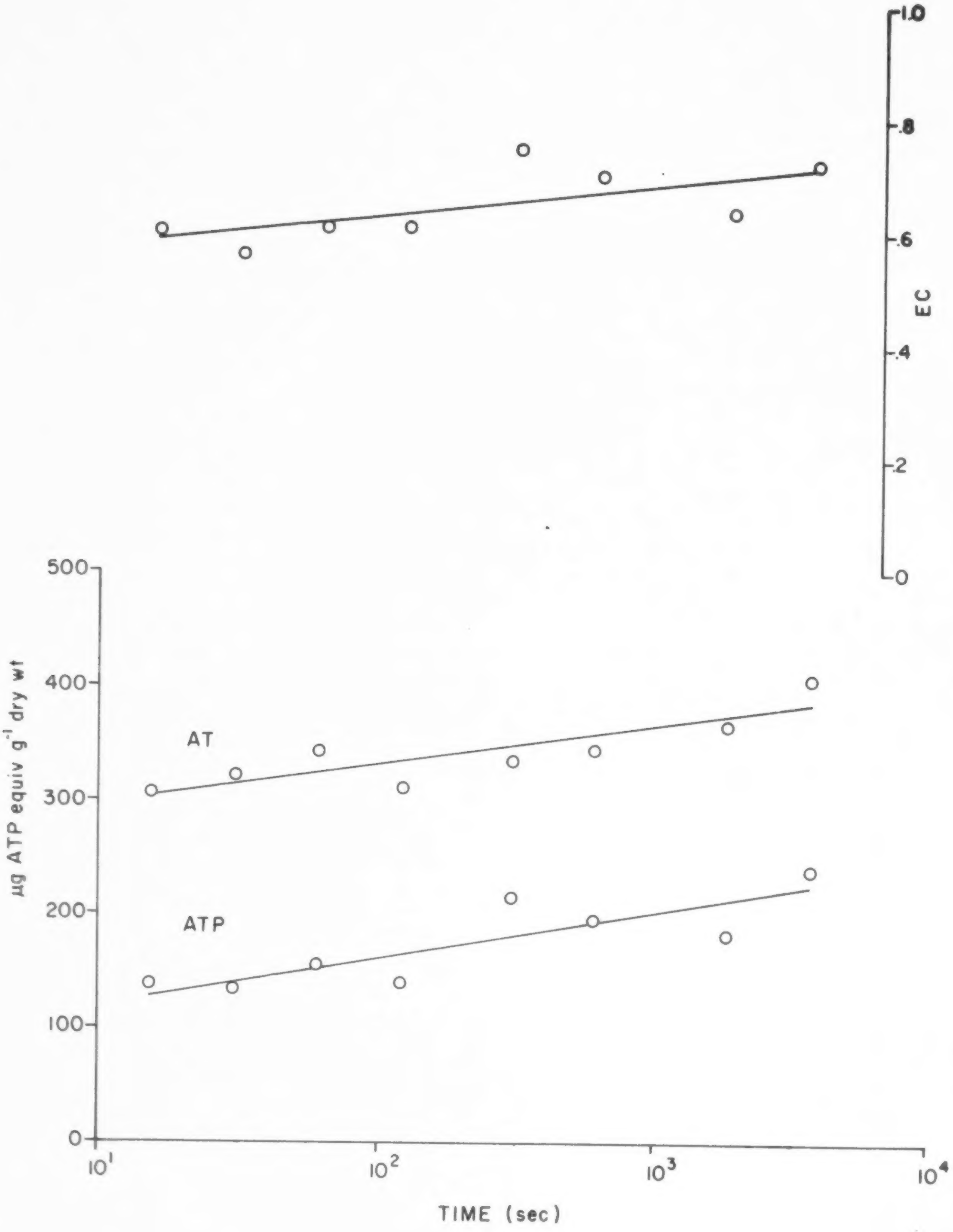


Figure 7.5. Semi-log regressions of ATP, AT, and EC vs. harvest-freeze delay interval (n = 2).

Table 7.6. Results suggest that adenylate concentrations reflect *in situ* levels for a period ≤2 minutes following harvest.

Adenylate levels in plants harvested during the day or at night, each at two delay intervals, appear in Table 7.7. Results suggest that increases in ATP, AT, and EC (as delay period lengthens) are light-related, since corresponding increase was not observed at night. It is of interest to note that EC was higher at night than during the day for the 30 sec delay.

Fresh-chopped vs. frozen-lyophilized-ground tissue is compared in Table 7.8. Although data show no significant difference, variability (i.e. standard error) in the lyophilized tissue was considerably lower, reflecting increased homogeneity of the quick-frozen, lyophilized, and more finely ground tissue. Fresh tissue was held (4 hrs) in river water at *in situ* temperature and light levels prior to processing.

The effect of epiphytes was evaluated with scraped vs. unscraped lyophilized leaves (Table 7.9). ATP, ADP, AT, and EC were significantly lower in unscraped tissue than in scraped tissue. The decreases was apparently due to low epiphyte adenylate levels.

Two modes of sample storage are evaluated in Table 7.10. Desiccated-dark storge of frozen-lyophilized-ground tissue and frozen extract storage were both suitable over 5 days, but not 20 days. AT significantly decreased in both preparations over a 20 day storage period.

Extraction--Four extractants are compared in Table 7.11. The superiority of boiling 1 mM EDTA + 5% PVPP (pH 7.6) was evident, among those extractants tested. Without addition of PVPP to the EDTA solution, light output was reduced and firefly reaction kinetics did not display their characteristic decay pattern (Figure 7.6). Data on recovery of added adenylates (internal standard) appear in Table 7.12. Again, the superiority of boiling 1 mM EDTA + 5% PVPP (pH 7.6) was evident. Standards, prepared in EDTA, quenched light output to a lesser extent than those prepared in either distilled water or neutralized acid (Table 7.13).

Duration of three extraction times is evaluated in Table 7.14. No significant differences were observed for extraction times of 5, 30, or 120 sec.

Extraction of individual plants vs. extraction of multiple aliquots from a pooled sample was compared in Table 7.15. Adenylates show no significant difference, although variability (i.e. standard error) in the pooled plant sample is considerably lower, as would be expected. Pooling masked natural variability between plants but yielded mean adenylate levels, similar to those obtained from individually extracted plants. Standard errors, associated with individually extracted plants, provide information on adenylate variability between plants in the field.

Conversion--Methodology experiments in the conversion procedure were tested by calculating conversion efficiency of AMP and ADP (internal standard)

TABLE 7.6. SEMI-LOG REGRESSION (N=8) STATISTICS FOR HARVEST-FREEZE DELAY

Statistic	ATP	AT	EC
Slope	.3991	.3431	.0559
Intercept	.8294	2.6321	.5188
Pearson Correlation Coefficient	.8507*	.8729*	.7078*

* $P < .05$

TABLE 7.7. EFFECT OF DAY VS NIGHT HARVEST, AT TWO FREEZE DELAY INTERVALS, ON ADENINE NUCLEOTIDES (μg ATP equiv g^{-1} dry wt) and EC (n=4)

Variable	Day (1200 hrs)		Night (2400 hrs)	
	30 sec	10 min	30 sec	10 min
ATP	$173^{a1} \pm 4^{2}$	$227^{b} \pm 4$	$169^{a} \pm 6$	$167^{a} \pm 3$
ADP	$92^{a} \pm 3$	$84^{a} \pm 4$	$53^{b} \pm 3$	$59^{b} \pm 1$
AMP^{3}	$52^{a} \pm 1$	$34^{a} \pm 4$	$33^{a} \pm 1$	$35^{a} \pm 2$
AT	$317^{a} \pm 5$	$344^{b} \pm 8$	$254^{c} \pm 8$	$260^{c} \pm 3$
EC	$.70^{a} \pm <.01$	$.78^{b} \pm .01$	$.77^{bc} \pm .01$	$.76^{c} \pm .01$

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

[3] Although the Kruskal-Wallis test shows a signifincant difference, the nonparametric multiple range test failed to detect differences between any pair of means for AMP.

TABLE 7.8. EFFECT OF TISSUE STATE ON ADENINE NUCLEOTIDES (μg ATP equiv g^{-1} dry wt) and EC (n=4)

Variable	Fresh-Chopped (5 mm)	Frozen-Lyophylized-Ground (425)
ATP	$226^{a1} \pm 21^{2}$	$253^{a} \pm 4$
ADP	$192^{a} \pm 36$	$151^{a} \pm 15$
AMP	$112^{a} \pm 14$	$129^{a} \pm 4$
AT	$530^{a} \pm 71$	$533^{a} \pm 20$
EC	$.61^{a} \pm .01$	$.62^{a} \pm .01$

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

TABLE 7.9. EFFECT OF EPIPHYTE REMOVAL, BY SCRAPING LYOPHILIZED LEAF TISSUE, ON ADENINE NUCLEOTIDES (μg ATP equiv g^{-1} dry wt) and EC (n=4)

Variable	Scraped Leaf	Unscraped Leaf	Scrapings[1] (Epiphytes)
ATP	$313^{a2} \pm 2^{3}$	$253^{b} \pm 1$	43 ± 2
ADP	$91^{a} \pm 1$	$84^{b} \pm 1$	33 ± 1
AMP	$106^{a} \pm 5$	$95^{a} \pm 4$	25 ± 1
AT	$509^{a} \pm 7$	$432^{b} \pm 3$	101 ± 2
EC	$.71^{a} \pm .01$	$.68^{b} \pm .01$	.59 ± .01

[1] Scrapings excluded from comparison test.

[2] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[3] Standard error.

TABLE 7.10. EFFECT OF TWO STORAGE METHODS AT 5 AND 20 DAYS ON ADENINE NUCLEOTIDES (μg ATP equiv g^{-1} dry wt) and EC (n=4)

Variable	Initial	5 Days		20 Days	
		Frozen-Lyophilized-Ground and Desiccated-Dark	Frozen Extract (-20°C)	Frozen-Lyophilized-Ground and Desiccated-Dark	Frozen Extract (-20°C)
ATP	278^{a}[1] ± 4[2]	267^{a} ± 3	277^{a} ± 4	248^{b} ± 3	272^{a} ± 4
ADP	91^{a} ± 5	96^{a} ± 4	87^{ab} ± 2	88^{ab} ± 3	75^{b} ± 3
AMP	120^{a} ± 3	114^{ab} ± 2	112^{ab} ± 4	107^{b} ± 3	84^{c} ± 3
AT	489^{a} ± 10	477^{a} ± 4	475^{a} ± 8	443^{b} ± 6	430^{b} ± 10
EC	$.66^{a}$ ± <.01	$.66^{a}$ ± <.01	$.67^{a}$ ± .01	$.66^{a}$ ± <.01	$.72^{b}$ ± <.01

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

TABLE 7.11. EFFECT OF EXTRACTANT ON ADENINE NUCLEOTIDES (μg ATP equiv g^{-1} dry wt) and EC (n=4)

Variable	Boiling 1 mM EDTA + 5% PVPP (pH 7.6)	Boiling 1 mM EDTA (pH 7.6)	Boiling Distilled Water	0-4°C .6N H_2SO_4 + 1 mM EDTA (neutralized to pH 7.6-7.9 with NaOH after extraction)
ATP	$144^{a1} \pm 2^{2}$	$27^{b} \pm <1$	$31^{b} \pm 1$	$38^{b} \pm 4$
ADP	$102^{a} \pm 2$	$25^{b} \pm <1$	$23^{b} \pm 1$	$22^{b} \pm 2$
AMP	$108^{a} \pm 4$	$61^{b} \pm 1$	$29^{c} \pm 1$	$27^{c} \pm 5$
AT	$354^{a} \pm 6$	$113^{b} \pm 1$	$82^{c} \pm 1$	$87^{c} \pm 11$
EC	$.55^{a} \pm <.01$	$.35^{b} \pm <.01$	$.52^{c} \pm .01$	$.57^{a} \pm .01$

[1] Values with same letter superscripts (between treatments) do not differ significantly (P > .05).

[2] Standard error.

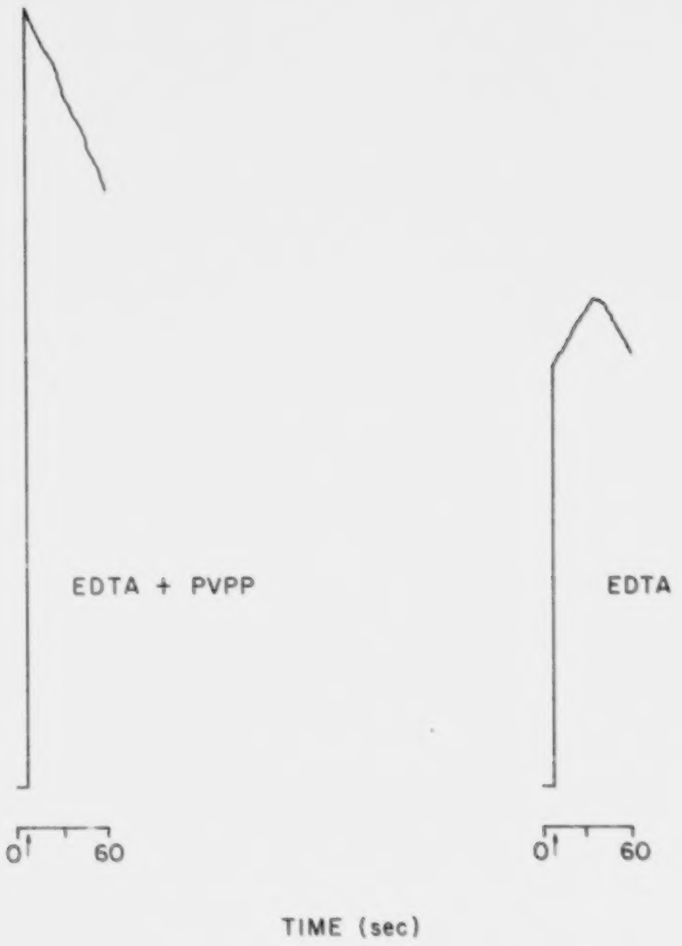


Figure 7.6. Reaction kinetics obtained from EDTA extraction of sample with and without PVPP addition. FLE is injected at time zero, the tube is vortexed, inserted into the photometer, and the shutter is opened (indicated by arrow).

TABLE 7.12. EFFECT OF EXTRACTANT ON RECOVERY (%) OF 200 NG ML^{-1} ATP, ADP,AMP ADDED IMMEDIATELY PRIOR TO EXTRACTION (N=4)

Variable	Boiling 1 mM EDTA + 5% PVPP (pH 7.6)	Boiling 1 mM EDTA (pH 7.6)	Boiling Distilled Water
ATP	82^{a}[1] ± 4[2]	17^{b} ± 1	22^{b} ± 2
ADP	83^{a} ± 22	31^{b} ± 4	25_{b} ± 5
AMP	112^{a} ± 12	64^{b} ± 10	51^{b} ± 7

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

TABLE 7.13. EFFECT OF EXTRACTANT ON LIGHT OUTPUT (NET COUNTS) (N=2)

ATP Standard ($ng\ ml^{-1}$)	[ATP] Reagents			[ATP+ADP] Reagents			[ATP+ADP+AMP] Reagents		
	X	Y	Z	X	Y	Z	X	Y	Z
4000	75096	47370	13760	66377	38828	12841	53072	32408	12682
40	426	287	86	350	252	83	294	204	77

X = 1 mM EDTA (pH 7.6)
Y = Distilled Water
Z = .6N H_2SO_4 + 1 mM EDTA (neutralized)

TABLE 7.14. EFFECT OF EXTRACTION DURATION ON ADENINE NUCLEOTIDES (μg ATP equiv g^{-1} dry wt) and EC (n=4)

Variable	Extraction Duration (sec)		
	5	30	120
ATP	$165^{a1} \pm 4^{2}$	$178^{a} \pm 4$	$162^{a} \pm 8$
ADP	$142^{a} \pm 9$	$133^{a} \pm 4$	$141^{a} \pm 8$
AMP	$116^{a} \pm 11$	$144^{a} \pm 11$	$111^{a} \pm 6$
AT	$423^{a} \pm 5$	$455^{a} \pm 10$	$414^{a} \pm 22$
EC	$.56^{a} \pm .01$	$.54^{a} \pm .01$	$.57^{a} \pm <.01$

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

TABLE 7.15. EFFECT OF POOLING PLANTS ON ADENINE NUCLEOTIDES (μg ATP equiv g^{-1} dry wt) and EC (n=4)

Variable	Individual Plants	Pooled Plants
ATP	369^{a}[1] ± 11[2]	$372^{a} \pm 4$
ADP	$65^{a} \pm 5$	$62^{a} \pm 1$
AMP	$66^{a} \pm 4$	$68^{a} \pm 3$
AT	$499^{a} \pm 16$	$501^{a} \pm 6$
EC	$.80^{a} \pm .01$	$.81^{a} \pm <.01$

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

to ATP. Concentrations given for treatment levels refer to the conversion reaction mixture.

Three buffers are evaluated in Table 7.16. AMP and ADP conversion efficiencies show no significant difference among the three buffers. TRICINE yielded the highest light output (Table 7.17).

Conversion enzyme cofactors are compared in Table 7.18. Results indicate that $MgSO_4$ is abolutely required, but that K_2SO_4 is not. Furthermore, K_2SO_4 addition may decrease conversion accuracy. $MgSO_4$, K_2SO_4, and higher pH all quenched light emission (Table 7.19).

The effect of heat deactivation on AMP and ADP conversion was assessed in Table 7.20. It is clear that this procedural step was essential. Without heat deactivation, ATP was produced in the presence of PEP and PK, presumably from ADP contained within the crude firefly lantern extract (Figure 7.7). With heat deactivation, ATP was not produced, and firefly reaction kinetics displayed their characteristic decay pattern.

Firefly lantern extract preparation--Three solutions to reconstitute lyophilized firefly lantern extract (FLE) are compared in Table 7.21. One vial of Sigma FLE-50 was trisected by weight to minimize FLE variability. Specified $MgSO_4$ concentration is exogenous, since Sigma FLE-50 also contains $MgSO_4$. The buffer solution at pH 8.1 resulted in significantly lower AMP and AT than either of the other reconstituents tested.

FLE aging times and temperatures are evaluated in Table 7.22. Sample extract was frozen between 6 and 24 hr assays, in order to minimize adenylate degradation. Although the 24 hr ATP levels were significantly higher than the 6 hr levels, the magnitude of the increase was slight. Significance resulted from the low variability within treatments. No other adenylate differences were observed. As both aging time and temperature increase, light output was reduced (Table 7.23).

The effect of guanosine diphosphate (GDP) addition to FLE was examined. ATP may be produced from ADP in the presence of guanosine triphosphate (GTP), or any other nucleoside triphosphate (NTP), and nucleoside diphosphokinase (NDPK). Results show no difference in adenylate levels (Table 7.24), however firefly reaction kinetics differ markedly (Figure 7.8). With GDP addition, light output was reduced and decay was more rapid in both standards and samples.

Photometry--Three photometer counting modes are evaluated in Table 7.25. Although ATP levels differed significantly among the three modes, the magnitudes of these differences were not large. No other adenylate differences were observed. Log-log standard regressions, derived from the three counting modes, were compared for ATP Reagents (Table 7.26). Slopes show no difference, but intercepts were significantly higher for the 30 sec integration. Correlation coefficients were highly significant. These regressions are plotted in Figure 7.9.

TABLE 7.16. EFFECT OF BUFFER ON AMP AND ADP CONVERSION EFFICIENCY (%), USING 80 NG ML^{-1} ATP,ADP,AMP (N=3)

Variable	15 mM TRICINE + 6 mM $MgSO_4$ (pH 7.6)	15 mM HEPES + 6 mM $MgSO_4$ (pH 7.6)	15 mM TRIS-HCl + 6 mM $MgSO_4$ (pH 7.6)
ADP	$106^{a1} \pm 2^{2}$	$103^{a} \pm 2$	$104^{a} \pm 3$
AMP	$83^{a} \pm 5$	$75^{a} \pm 5$	$84^{a} \pm 4$

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

TABLE 7.17. EFFECT OF BUFFER ON LIGHT OUTPUT (NET COUNTS) (N=1)

ATP Standard (ng ml^{-1})	[ATP] Reagents			[ATP+ADP] Reagents			[ATP+ADP+AMP] Reagents		
	X	Y	Z	X	Y	Z	X	Y	Z
4000	59163	54416	53769	46227	45020	43242	38650	37885	37091
40	367	343	323	303	283	269	294	264	258

X = 15 mM TRICINE + 6 mM $MgSO_4$ (pH 7.6)
Y = 15 mM HEPES + 6 mM $MgSO_4$ (pH 7.6)
Z = 15 mM TRIS-HCl + 6 mM $MgSO_4$ (pH 7.6)

TABLE 7.18. EFFECT OF ENZYME COFACTORS ON AMP AND ADP CONVERSION EFFICIENCY (%), USING 80 NG ML^{-1} ATP,ADP,AMP (N=3)

Variable	15 mM TRICINE (pH 7.6)	15 mM TRICINE + 6 mM $MgSO_4$ (pH 7.6)	15 mM TRICINE + 6 mM $MgSO_4$ + 7.5 mM K_2SO_4 (pH 7.6)	15 mM TRICINE + 6 mM $MgSO_4$ + 7.5 mM K_2SO_4 (pH 8.1)
ADP	-1^{a}[1] ± 1[2]	108^{b} ± 4	114^{b} ± 2	114^{b} ± 1
AMP	-1^{a}[3] ± 1	110^{b} ± 3	85^{ab} ± <1	82^{ab} ± 4

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

[3] Group 1 shows no difference with groups 3 and 4 for AMP conversion, because the nonparametric multiple range test uses ranks.

TABLE 7.19. EFFECT OF ENZYME COFACTORS ON LIGHT OUTPUT (NET COUNTS) (N=2)

ATP Standard (ng ml^{-1})	[ATP] Reagents				[ATP+ADP] Reagents				[ATP+ADP+AMP] Reagents			
	W	X	Y	Z	W	X	Y	Z	W	X	Y	Z
4000	48753	45351	33145	28813	38247	37239	27919	24775	30355	31460	23637	19925
40	283	270	177	152	217	209	147	123	166	172	126	11[illegible]

W = 15 mM TRICINE (pH 7.6)
X = 15 mM TRICINE + 6 mM $MgSO_4$ (pH 7.6)
Y = 15 mM TRICINE + 6 mM $MgSO_4$ + 7.5 mM K_2SO_4 (pH 7.6)
Z = 15 mM TRICINE + 6 mM $MgSO_4$ + 7.5 mM K_2SO_4 (pH 8.1)

TABLE 7.20. EFFECT OF HEAT DEACTIVATION ON AMP AND ADP CONVERSION EFFICIENCY (%), USING 80 NG ML^{-1} ATP,ADP,AMP (N=4)

Variable	Heat (2 min, 100°C)	No Heat
ADP	$111^{a1} \pm 4^{2}$	$55^{b} \pm 13$
AMP	$102^{a} \pm 7$	$185^{b} \pm 14$

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

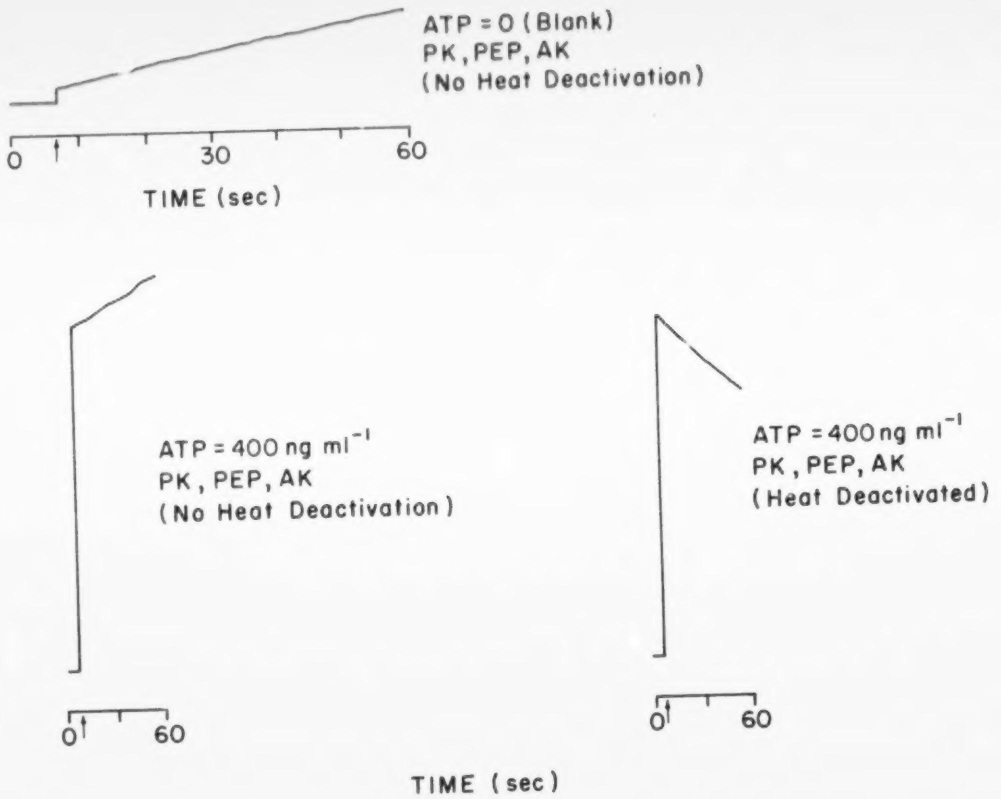


Figure 7.7. Reaction kinetics with and without heat deactivation. FLE is injected at time zero, the tube is vortexed, inserted into the photometer, and the shutter is opened (indicated by arrow).

TABLE 7.21. EFFECT OF FLE RECONSTITUENT ON ADENINE NUCLEOTIDES (μg ATP equiv g^{-1} dry wt) and EC (n=4)

Variable	Distilled Water	45 mM TRICINE + 18 mM $MgSO_4$ (pH 7.6)	45 mM TRICINE + 18 mM $MgSO_4$ (pH 8.1)
ATP	$92^{a1} \pm 1^{2}$	$93^{a} \pm 1$	$92^{a} \pm 1$
ADP	$65^{a} \pm 1$	$66^{a} \pm 2$	$62^{a} \pm 1$
AMP	$65^{a} \pm 3$	$67^{a} \pm 3$	$54^{b} \pm 1$
AT	$221^{a} \pm 4$	$226^{a} \pm 2$	$208^{b} \pm 1$
EC	$.57^{a} \pm .01$	$.56^{a} \pm .01$	$.59^{b} \pm <.01$

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

TABLE 7.22. EFFECT OF FLE AGING TIME AND TEMPERATURE ON ADENINE NUCLEOTIDES
(µg ATP equiv g^{-1} dry wt) and EC (n=4)

Variable	6 hr		24 hr	
	4°C	25°C	4°C	25°C
ATP	88^{a}[1] ± 1[2]	89^{a} ± 1	91^{b} ± 1	93^{b} ± 1
ADP	72^{a} ± 1	70^{a} ± 2	67^{a} ± 3	68^{a} ± 2
AMP	48^{a} ± 4	60^{a} ± 1	56^{a} ± 3	55^{a} ± 3
AT	208^{a} ± 3	218^{a} ± 2	214^{a} ± 2	215^{a} ± 2
EC	$.60^{a}$ ± .01	$.57^{a}$ ± <.01	$.58^{a}$ ± .01	$.59^{a}$ ± <.01

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$)

[2] Standard error.

TABLE 7.23. EFFECT OF FLE AGING TIME AND TEMPERATURE ON LIGHT OUTPUT (NET COUNTS) (N=2)

ATP Standard ($ng\ ml^{-1}$)	[ATP] Reagents				[ATP+ADP] Reagents				[ATP+ADP+AMP] Reagents			
	W	X	Y	Z	W	X	Y	Z	W	X	Y	Z
4000	87610	76269	75334	46857	63902	50851	50782	31777	50775	40664	42445	25405
40	536	388	407	266	384	271	283	187	306	209	224	146

W = 6 hr, 4°C
X = 6 hr, 25°C
Y = 24 hr, 4°C
Z = 24 hr, 25°C

TABLE 7.24. EFFECT ON GDP ADDITION TO FLE ON ADENINE NUCLEOTIDES
(μg ATP equiv g^{-1} dry wt) and EC
(n=4)

Variable	No GDP	6.85 μg ml^{-1} GDP
ATP	$104^{a1} \pm 1^{2}$	$104^{a} \pm 2$
ADP	$62^{a} \pm 2$	$65^{a} \pm 6$
AMP	$35^{a} \pm 2$	$38^{a} \pm 1$
AT	$202^{a} \pm 2$	$206^{a} \pm 1$
EC	$.67^{a} \pm .01$	$.66^{a} \pm <.01$

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

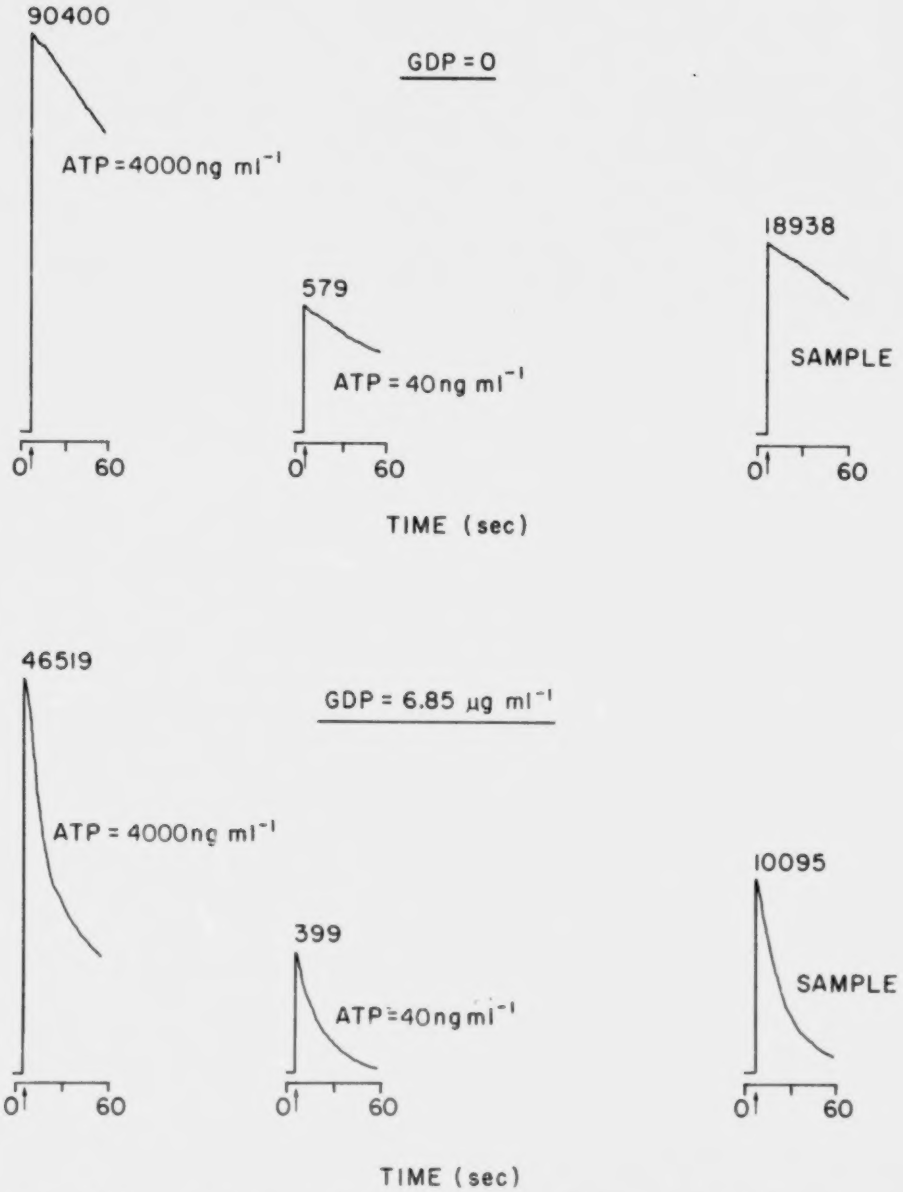


Figure 7.8. Reaction kinetics with and without GDP addition. FLE is injected at time zero, the tube is vortexed, inserted into the photometer, and the shutter is opened (indicated by arrow). Counts represent a 10 sec integration period, immediately following a 10 sec delay from time zero.

TABLE 7.25. EFFECT OF PHOTOMETER COUNTING MODE ON ADENINE NUCLEOTIDES
(μg ATP equiv g^{-1} dry wt) and EC (n=4)

Variable	10 Sec Delay followed by:		Peak Height
	10 sec Integral	30 sec Integral	
ATP	162^{a}[1] ± 2[2]	171^{b} ± 2	150^{c} ± 2
ADP	161^{a} ± 7	145^{a} ± 4	141^{a} ± 6
AMP	230^{a} ± 2	235^{a} ± 17	238^{a} ± 7
AT	552^{a} ± 5	551^{a} ± 14	529^{a} ± 7
EC	.44^{a} ± <.01	.44^{a} ± .01	.42^{a} ± <.01

[1] Values with same letter superscripts (between treatments) do not differ significantly (P > .05).

[2] Standard error.

TABLE 7.26. COMPARISON OF LOG-LOG REGRESSION (N=4) STATISTICS, OBTAINED FROM THREE PHOTOMETER COUNTING MODES WITH [ATP] REAGENTS

Statistic	10 Sec Delay followed by:		Peak Height
	10 Sec Integral	30 Sec Integral	
Slope	1.1150^{a1}	1.1289^{a}	1.0699^{a}
Intercept	10.9392^{a}	11.4838^{b}	10.6016^{a}
Pearson Correlation Coefficient	.9989*	.9985*	.9999*

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

* $P < .001$

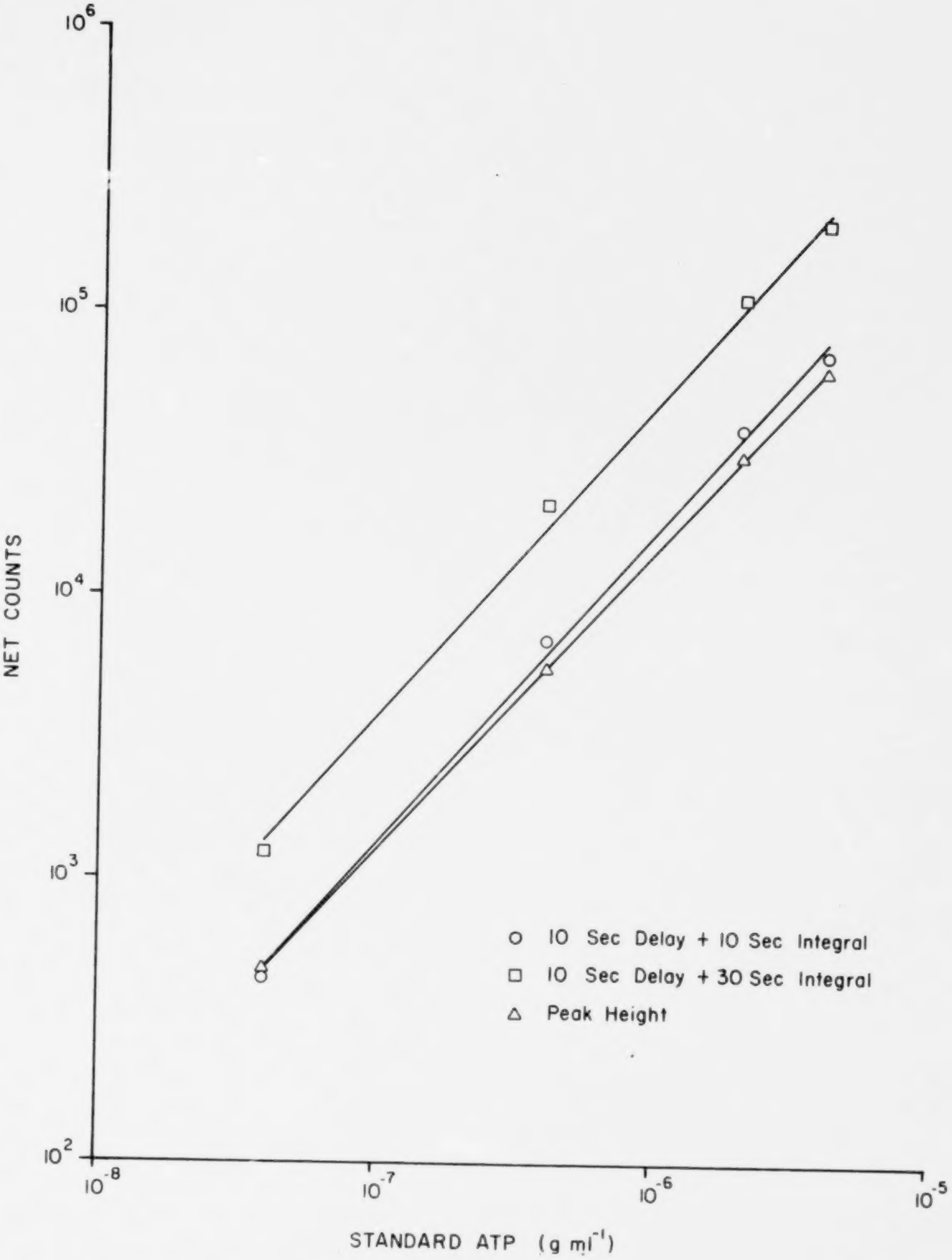


Figure 7.9. Comparison of photometer counting modes with ATP reagents (n = 2).

Mixing kinetics with the peak height mode, using a 6 x 50 mm tube, were examined by varying the ATP standard/FLE volume ratio (Table 7.27). Proper mixing was evaluated, as described in Figure 7.10. Although several standard/FLE volume ratios mixed properly (i.e. 20/15, 50/15, 100/50), samples would not consistently mix well. Therefore, whenever peak height was used, proper mixing was evaluated.

Standard curve--Six standards and one blank were routinely run for each reaction Tube A, B, and C. Using a 10 sec delay followed by a 10 sec integration, representative standards, net mean counts, and regression-calculated ATP concentrations appear in Table 7.28. Log-log regression plots are shown in Figure 7.11. The associated statistics are presented in Table 7.29. Correlation coefficients were highly significant. Differential quenching of light output was apparent among the three regressions.

Analytical variability--Optimized recovery and conversion efficiencies were presented in Table 7.30. Since these efficiencies were near 100% with relatively low variability (i.e. small standard error), no correction factors were applied in data reduction.

Photometer variability, expressed as coefficient of variation, appears in Table 7.31. Coefficients were generally <2%, with the exception of blank readings. Higher coefficients for blanks were the mathematical result of division by a small mean rather than multiplication by a large standard deviation. These data were based on a 10 sec delay, followed by a 10 sec integration.

Tissue Comparisons--

Zostera marina--Adenylate levels in four types of tissues from Z. marina are presented in Table 7.32. Leaf tissue clearly had the highest level of ATP, ADP, AT, and EC, while root plus rhizome tissue showed the lowest measured levels of ATP, ADP, AMP, and AT. An adenylate correlation matrix was derived by pooling values from all four tissues (Table 7.33). ATP was positively correlated with ADP, AT, and EC, while ADP was positively correlated with AT and EC. Environmental and morphometric data, associated with this eelgrass sample, are presented in Table 7.34.

Other species--Adenylate levels in Z. marina epiphytes, aboveground Ruppia maritima (widgeongrass), and aboveground Spartina alterniflora (saltmarsh cordgrass) appear in Table 7.35 for comparative purposes. Z. marina leaf tissue and aboveground R. maritima had comparable adenylate concentrations. Both were higher than either the epiphytes or aboveground S. alterniflora tissue. Environmental data, associated with collection of these samples, are presented in Table 7.36.

Seasonal Survey--

Monthly mean aboveground adenylates (Figure 7.12), belowground adenylates (Figure 7.13), and resultant EC values (Figure 7.14) in Z. marina are plotted. Each of these time series contained significant differences ($P < .05$) over the one year period. Adenylates and EC were generally higher in aboveground tissue.

TABLE 7.27. EXAMINATION OF REAGENT MIXING IN PEAK HEIGHT MODE (COUNTS) (N=5)

ATP Standard Volume (µl)	Statistic	FLE Volume (µl)				
		15	25	50	100	200
20	$\bar{x}$	4532	8314	13310	8452	10038
	$s/\bar{x}$	.08	.11	.19	.28	.07
	% PM[1]	100	80	60	0	0
50	$\bar{x}$	3625	9776	19896	22097	26449
	$s/\bar{x}$	.04	.11	.06	.22	.15
	% PM	100	80	0	0	0
100	$\bar{x}$	1287	6077	24241	40259	38310
	$s/\bar{x}$	.33	.08	.03	.78	.13
	% PM	0	0	100	20	0

[1] Properly Mixed Tubes.

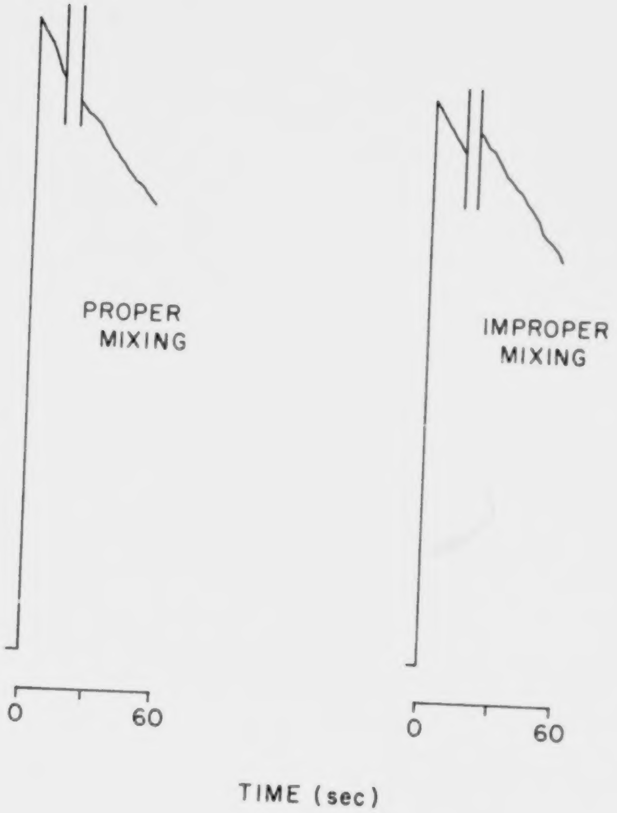


Figure 7.10. Mixing kinetics in peak height mode. The sample tube is inserted into the photometer, and FLE is injected at time zero with the electronic pipet system which simultaneously activates the photometer. After 15 sec, the tube is removed, vortexed, and re-inserted into the photometer. Continuity in decay kinetics indicates proper initial mixing.

TABLE 7.28. LOG-LOG REGRESSION STANDARDS, NET MEAN COUNTS (N=3), AND PREDICTED ATP CONCENTRATIONS, USING A 10 SEC DELAY FOLLOWED BY A 10 SEC INTEGRAL

Standard ATP ($ng\ ml^{-1}$)	[ATP] Reagents		[ATP+ADP] Reagents		[ATP+ADP+AMP] Reagents	
	Y	X	Y	X	Y	X
4000	89358	3703	69379	3719	58048	3735
2000	45435	1995	35333	2009	29552	2018
1000	22742	1059	17480	1057	14472	1052
400	8558	433	6477	427	5314	422
100	1719	100	1306	99	1089	99
40	596	38	462	38	385	38

Y = Net mean counts
X = Regression-calculated ATP ($ng\ ml^{-1}$)

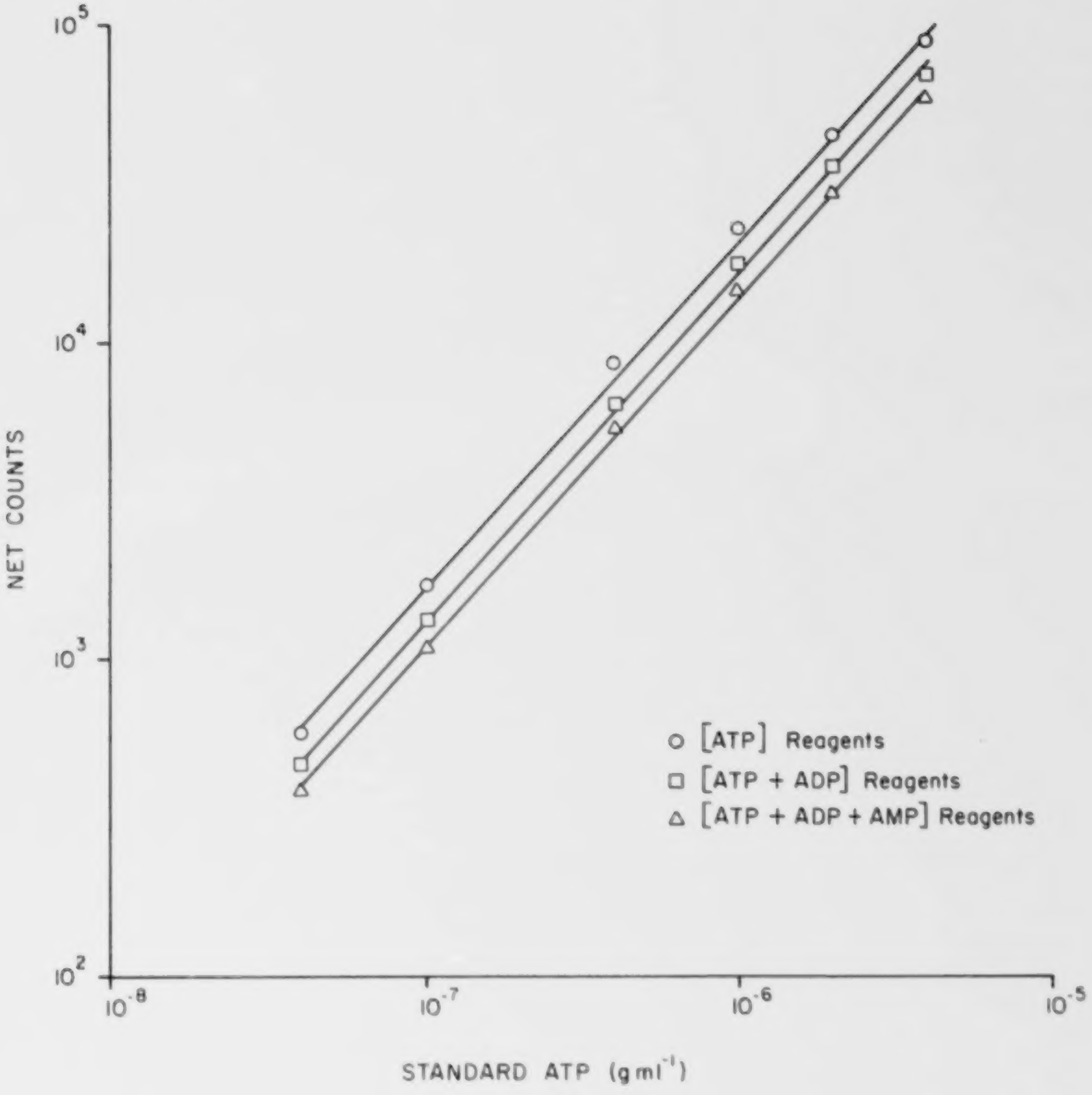


Figure 7.11. Standard curve regressions, using a 10 sec delay followed by a 10 sec integration (n = 3).

TABLE 7.29. LOG-LOG REGRESSION (N=6) STATISTICS, USING A 10 SEC DELAY FOLLOWED BY A 10 SEC INTEGRAL

Statistic	[ATP] Reagents	[ATP+ADP] Reagents	[ATP+ADP+AMP] Reagents
Slope	1.0932	1.0957	1.0963
Intercept	10.8890	10.7902	10.7144
Pearson Correlation Coefficient	.9994*	.9995*	.9996*

* $P < .001$

TABLE 7.30. RECOVERY AND CONVERSION EFFICIENCY (%) WITH OPTIMIZED METHOD (N=4)

Variable	Recovery: 200 ng ml^{-1} ATP,ADP,AMP added immediately prior to extraction	Conversion: ATP,ADP,AMP Standard (ng ml^{-1})	
		1000	80
ATP	109 ± 9[1]	-	-
ADP	96 ± 5	102 ± 1	104 ± 1
AMP	97 ± 4	108 ± 2	96 ± 2

[1] Standard error.

TABLE 7.31. PHOTOMETER VARIABILITY (COEFFICIENT OF VARIATION) WITH OPTIMIZED METHOD (N=5)

Standard ATP ($ng\ ml^{-1}$)	[ATP] Reagents	[ATP+ADP] Reagents	[ATP+ADP+AMP] Reagents
Blank	.250	.026	.057
4000	.010	.006	.010
2000	.006	.012	.005
1000	.010	.012	.015
400	.007	.008	.019
100	.010	.011	.016
40	.015	.022	.003

TABLE 7.32. ADENINE NUCLEOTIDES (G ATP EQUIV G^{-1} DRY WT) AND EC IN FOUR TYPES OF TISSUE FROM Z. MARINA (N=4)

Variable	Leaf	Leaf Sheath	Root + Rhizome	Seed Pod
ATP	245^{a}[1] ± 2[2]	72^{b} ± <1	34^{c} ± <1	129^{d} ± 3
ADP	95^{a} ± 1	49^{b} ± 2	13^{c} ± 1	63^{d} ± 2
AMP	47^{a} ± 4	55^{a} ± 3	27^{b} ± <1	108^{c} ± 14
AT	387^{a} ± 5	175^{b} ± 5	74^{c} ± 1	299^{d} ± 13
EC	$.76^{a}$ ± .01	$.55^{b}$ ± .01	$.55^{b}$ ± <.01	$.54^{b}$ ± .03

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

TABLE 7.33. SPEARMAN CORRELATION COEFFICIENTS AMONG ADENINE NUCLEOTIDES AND EC, OBTAINED BY POOLING VALUES FROM FOUR TISSUE TYPES (N=16)

	ADP	AMP	AT	EC
ATP	.9512*	.4490	.9608*	.6206*
ADP		.4240	.9594*	.5871*
AMP			.5018	-.2724
AT				.4682

* $P < .05$

TABLE 7.34. ENVIRONMENTAL DATA AND MORPHOMETRICS FOR *Z. MARINA*, USED IN TISSUE STUDY

1981 Harvest (mo)	May
Low Tide	
EST (hr)	1214
Height (m)	.1
Salinity (^{o}/oo)	22.58
pH	8.00
Water Temp. (°C)	23.8
38°N Daylength (hr-min)	14-15
Density (shoots m^{-2})	1333
Shoot Length (cm)	
$\bar{x}$ ± SE (n)	25.8 ± 1.4 (40)
Live Dry Wt (g m^{-2})	
Aboveground	291
Belowground	109
Total	400

TABLE 7.35. ADENINE NUCLEOTIDES (μG ATP EQUIV G^{-1} DRY WT) AND EC IN *Z. MARINA* EPIPHYTES (N=4), ABOVEGROUND *RUPPIA MARITIMA* (N=2), AND ABOVEGROUND *SPARTINA ALTERNIFLORA* (N=4)

Variable	Epiphytes	*R. maritima*	*S. alterniflora*
ATP	43 ± 2[1]	215 ± 5	87 ± 1
ADP	33 ± 1	137 ± <1	69 ± 1
AMP	25 ± 1	41 ± 8	33 ± 1
AT	101 ± 2	394 ± 3	189 ± 2
EC	.59 ± .01	.72 ± .02	.64 ± <.01

[1] Standard error.

TABLE 7.36. ENVIRONMENTAL DATA FOR COLLECTION OF EPIPHYTES, *R. MARITIMA*, AND *S. ALTERNIFLORA*

Variable	Epiphytes	*R. maritima*	*S. alterniflora*
1981 Harvest (mo)	Jul	Jun	Apr
Low Tide			
EST (hr)	0951	1336	1702
Height (m)	.1	-.1	0
Salinity (°/oo)	20.87	20.42	22.89
pH	7.86	8.12	8.02
Water Temp. (°C)	28.0	27.1	19.5
38°N Daylength (hr-min)	14-39	14-47	13-24

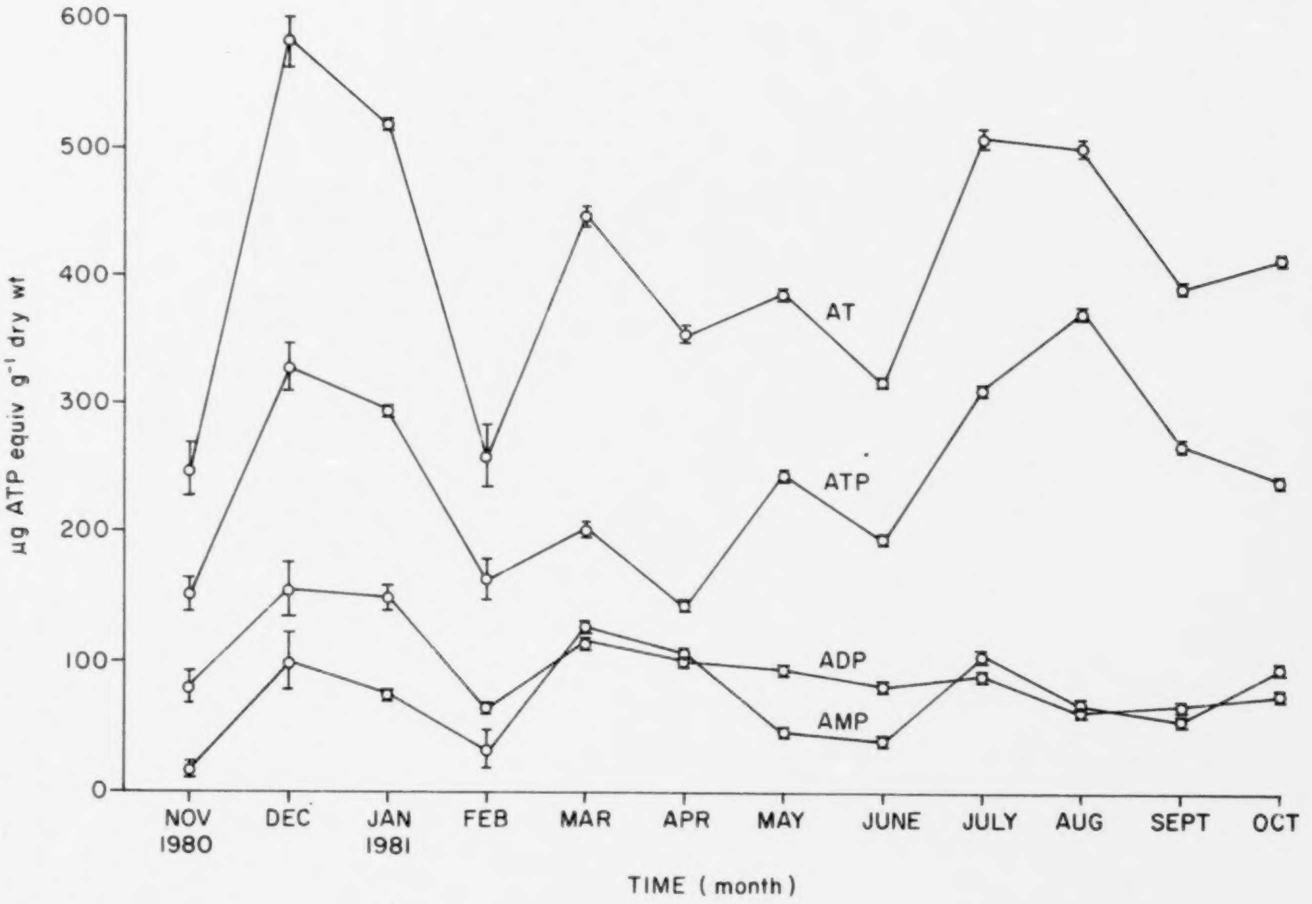


Figure 7.12. Monthly adenine nucleotides from aboveground Z. marina (n=4). Error bars are 1 standard error.

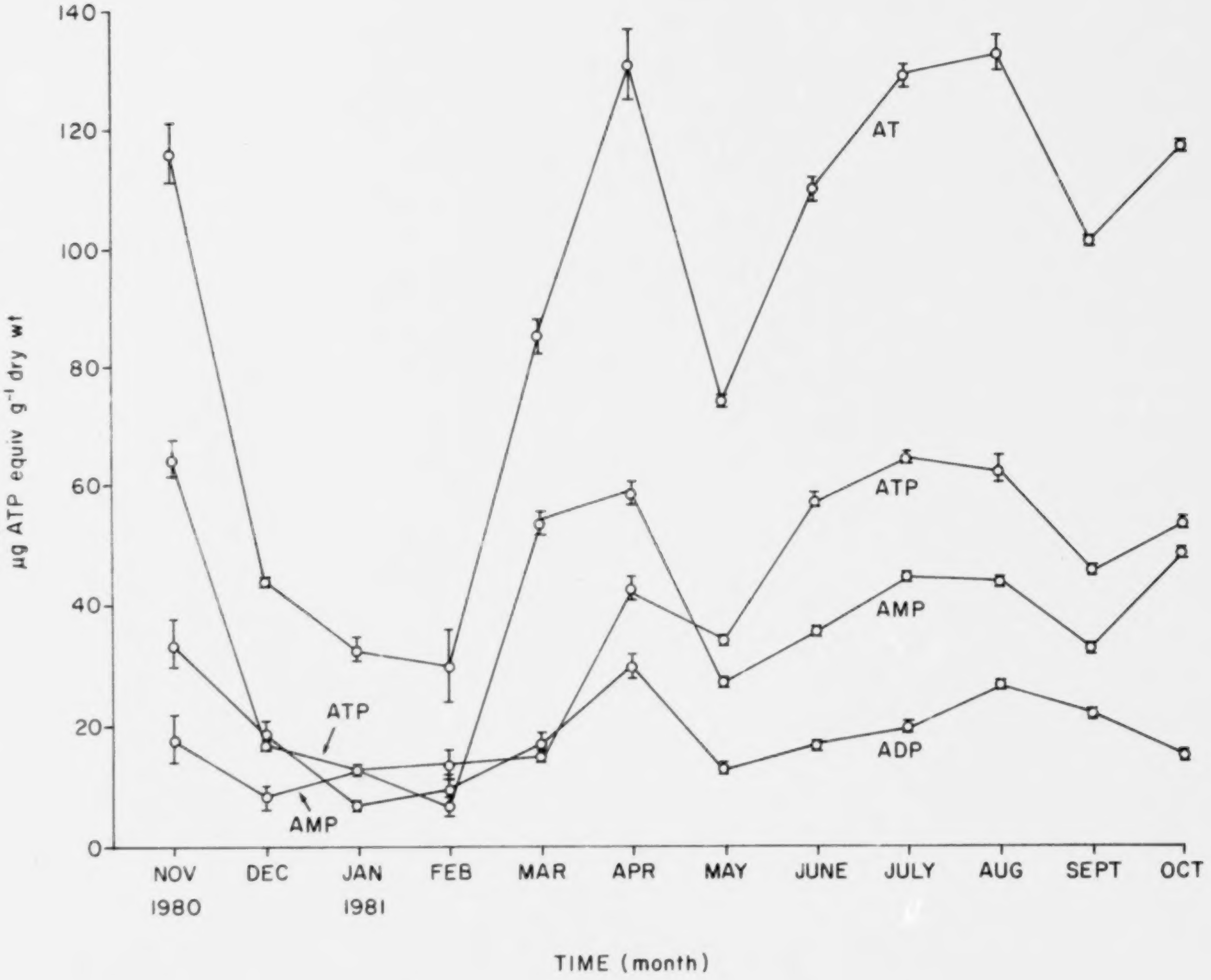


Figure 7.13. Monthly adenine nucleotides from belowground Z. marina (n=4). Error bars are 1 standard error.

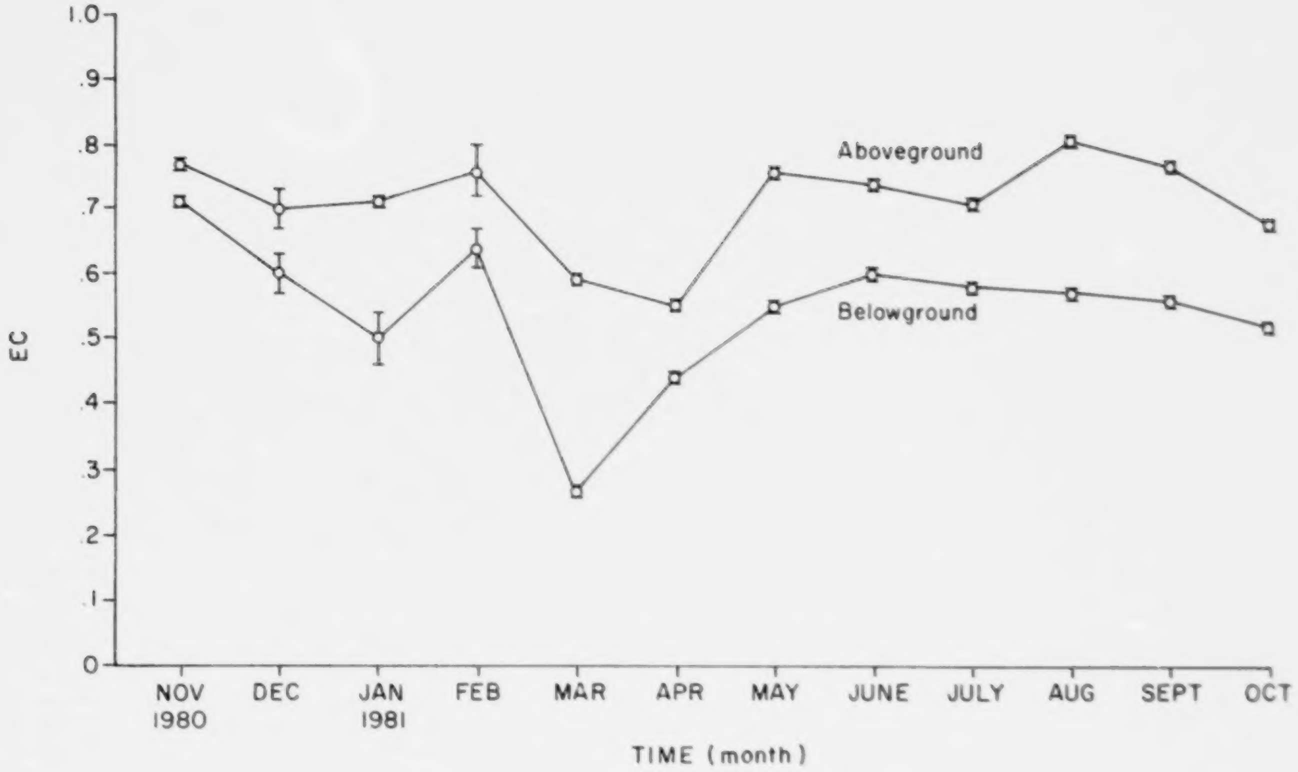


Figure 7.14. Monthly EC from above and belowground Z. marina (n=4). Error bars are 1 standard error.

Correlation matrices for aboveground (Table 7.37) and belowground (Table 7.38) adenylates were derived by pooling values from all 12 months. For both above and belowground adenylates, AT was positively correlated with ATP, ADP, and AMP, while EC was negatively correlated with AMP. Correlation coefficients between above and belowground adenylates, using monthly means, are presented in Table 7.39. Above and belowground AMP were positively correlated. Weaker positive correlation ($0.05<P<0.10$) existed between above and belowground EC.

Environmental data appear in Table 7.40 and Figure 7.15. Morphometric data are plotted in Figure 7.16. Maxima for biomass, number of shoots, and shoot length occurred in May, June, and July, respectively.

Aboveground and belowground adenylate correlations with environmental and morphometric data, using monthly means, are presented in Tables 7.41 and 7.42, respectively. No significant correlations were observed for aboveground adenylates. Although several belowground adenylate correlations were significant, no clear patterns were evident.

Discussion

Adenine Nucleotide Methodology Experiments--

Sample collection and preparation--The logistical problem of sampling a submerged aquatic macrophyte, so that adenylates are maintained at *in situ* levels, was minimized by freezing plants in liquid nitrogen within 2 min after harvest. An increase in light level, associated with the harvest procedure, appears to be responsible for the observed elevation in ATP, AT, and EC. Transitions from dark to light result in rapid chloroplastic ATP increase (Hampp et al., 1982; Cockburn, 1974; Santarius and Heber, 1965), presumably by photophosphorylation. An accompanying elevation in cytoplasmic ATP reflects intracellular adenylate transfer (Sellami, 1976).

Rapid freezing with liquid nitrogen (<3 min) is more effective than slow freezing with dry ice (5-15 min) at preserving *in situ* adenylate levels in *Spartina patens* leaves (Mendelssohn and McKee, 1981). The longer time interval required for dry ice freezing may allow for more transphosphorylase and ATPase activity. Even after plant tissue is frozen, enzymic activity persists (Bieleski, 1964).

Lyophilization of frozen tissue (e.g. Bomsel and Sellami, 1974; Wilson, 1978) effectively maintained *in situ* adenylate levels, and homogenization by grinding lowered variability in replicate aliquots. Advantages of lyophilization include adenylate stabilization by enzyme deactivation (dehydration) and direct determination of tissue dry weight (Mendelssohn and McKee, 1981). It is critical that the sample remain frozen below its lowest eutectic point during the time interval required by the lyophilizer to reach sufficient vacuum. Freeze-thaw treatment increases cell permeability to ATP (Rhodes and Stewart, 1974) and may dislodge ATPases from thylakoid membranes (Garber and Steponkus, 1976), reducing ATP content in plant tissue, (Mendelssohn and McKee, 1981).

TABLE 7.37. SPEARMAN CORRELATION COEFFICIENTS AMONG ADENINE NUCLEOTIDES AND EC, FROM ABOVEGROUND _Z. MARINA_ USED IN SEASONAL SURVEY, OBTAINED BY POOLING ALL VALUES (N=48)

	ADP	AMP	AT	EC
ATP	.0943	.2622	.8475*	.2641
ADP		.4282*	.4806*	-.5100*
AMP			.6121*	-.7952*
AT				-.2106

* $P < .05$

TABLE 7.38. SPEARMAN CORRELATION COEFFICIENTS AMONG ADENINE NUCLEOTIDES AND EC, FROM BELOWGROUND _Z. MARINA_ USED IN SEASONAL SURVEY, OBTAINED BY POOLING ALL VALUES (N=48)

	ADP	AMP	AT	EC
ATP	.6150*	.3280*	.8416*	.3846*
ADP		.3414*	.7160*	.1548
AMP			.7078*	-.6160*
AT				-.0263

* P < .05

TABLE 7.39. SPEARMAN CORRELATION COEFFICIENTS BETWEEN ABOVE AND BELOWGROUND ADENINE NUCLEOTIDES AND EC, FROM _Z. MARINA_ OBTAINED IN SEASONAL SURVEY, USING MONTHLY MEANS (N=12)

Variable	Correlation Coefficient
ATP	.0420
ADP	-.1961
AMP	.6364*
AT	-.0490
EC	.5845

* $P < .05$

TABLE 7.40. MONTHLY ENVIRONMENTAL DATA FOR COLLECTION OF *Z. MARINA*, USED IN SEASONAL SURVEY

Harvest (mo)	Low Tide		38°N Daylength (hr-min)
	EST (hr)	Height (m)	
Nov 1980	1022	.1	10-5
Dec	0855	0	9-31
Jan 1981	1730	-.2	9-44
Feb	1706	-.2	10-16
Mar	1301	-.1	11-23
Apr	1359	-.1	12-37
May	1214	.1	14-15
Jun	1336	-.1	14-47
Jul	0951	.1	14-39
Aug	1604	0	14-10
Sept	0603	.1	12-56
Oct	0727	.2	11-44

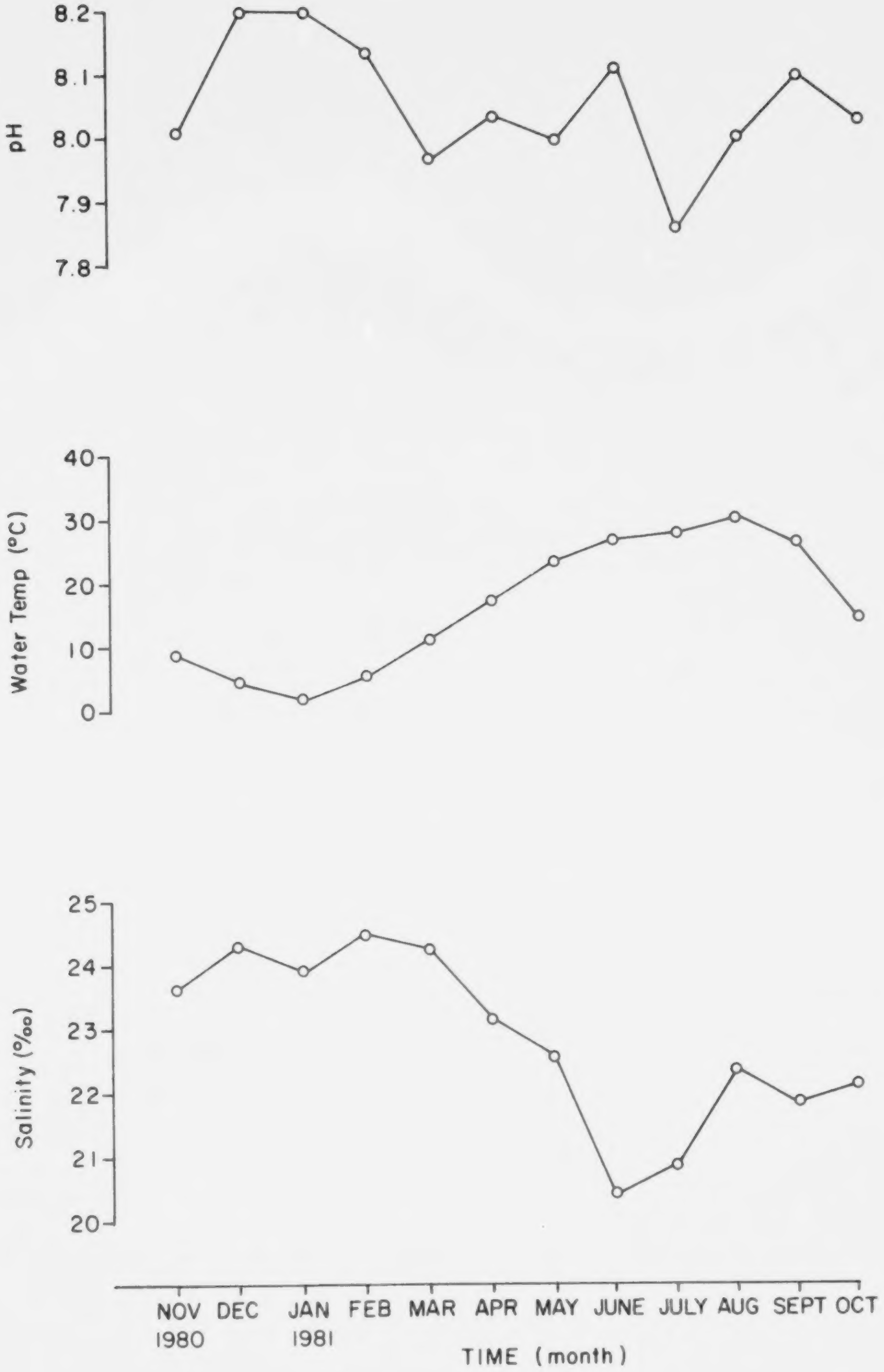


Figure 7.15. Monthly environmental data for collection of *Z. marina*, used in seasonal survey.

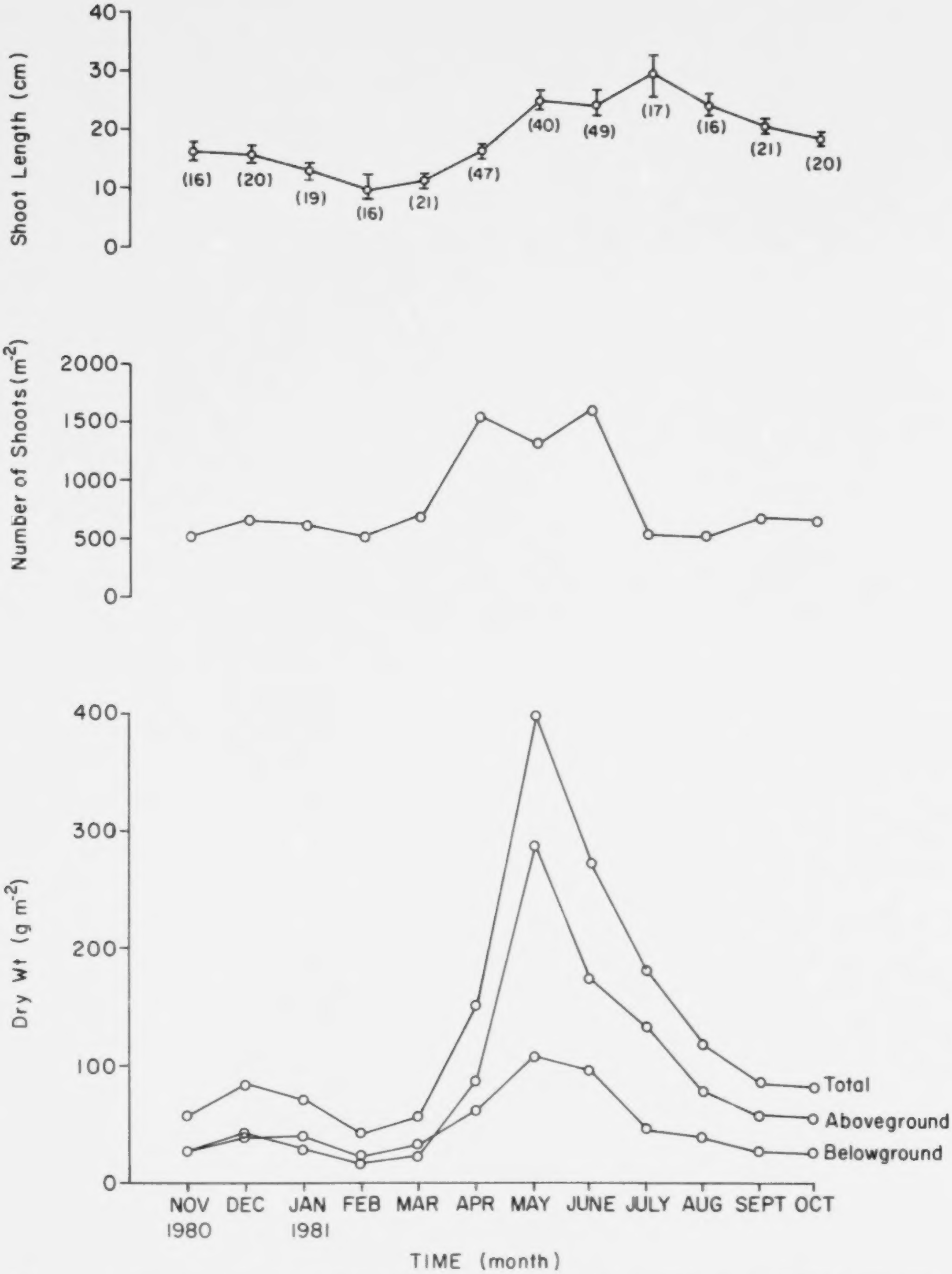


Figure 7.16. Monthly morphometrics for Z. marina, used in seasonal survey. Shoot length error bars are 1 standard error and numbers in parentheses are n.

TABLE 7.41. SPEARMAN CORRELATION COEFFICIENTS OF *Z. MARINA* ABOVEGROUND ADENINE NUCLEOTIDES AND EC WITH ENVIRONMENTAL AND MORPHOMETRIC DATA, OBTAINED IN SEASONAL SURVEY, USING MONTHLY MEANS (N=12)

	Low Tide Height	Salinity	pH	Water Temp.	Daylength	Shoot Density	Shoot Length	Live Dry Wt		
								Aboveground	Belowground	Total
ATP	.2087	-.1538	-.0526	.2168	.0559	-.2686	.3636	.1958	.1051	.2308
ADP	-.2627	.3427	.1825	-.4825	-.3566	.4064	-.2587	0	.5289	.0559
AMP	-.0432	.0559	-.2597	.0140	-.0559	.2686	-.0420	.0420	.1891	.0490
AT	.0144	.0559	.0175	-.0699	-.1888	-.1095	.0699	.0210	.1436	.0559
EC	.1917	-.1968	-.0494	.3234	.2109	-.4529	.3093	.0668	-.1919	.0738

* $P < .05$

TABLE 7.42. SPEARMAN CORRELATION COEFFICIENTS OF _Z. MARINA_ BELOWGROUND ADENINE NUCLEOTIDES AND EC WITH ENVIRONMENTAL AND MORPHOMETRIC DATA, OBTAINED IN SEASONAL SURVEY, USING MONTHLY MEANS (N=12)

	Low Tide Height	Salinity	pH	Water Temp.	Daylength	Shoot Density	Shoot Length	Live Dry Wt		
								Aboveground	Belowground	Total
ATP	.6417*	-.7075*	-.5272	.6900*	.5394	-.1558	.6970*	.4518	.0912	.4238
ADP	.3461	-.2557	-.3374	.4098	.1436	-.0726	.2907	.1646	.0281	.1856
AMP	.2303	-.4685	-.6175*	.5385	.4825	.4135	.3427	.3566	.1856	.3007
AT	.4390	-.6154*	-.5719	.7133*	.5175	.0106	.6014*	.4685	.1506	.4266
EC	.0991	.0070	.2127	0	-.0385	-.4726	.0525	-.0981	-.1737	-.0595

* P < .05

Removal of epiphytes from *Z. marina* leaf blades was essential in order to quantify adenine nucleotides in eelgrass tissue alone. Epiphytes can be a significant proportion of an aboveground tissue sample. For example, epiphytes comprise an average of 24% of the total eelgrass leaf plus epiphyte biomass (dry wt) in a North Carolina estuary (Penhale, 1977). Low epiphyte adenylate levels, relative to levels in *Z. marina* leaf tissue, may be attributed to the inclusion of small amounts of sediment, as well as siliceous diatom frustules, in epiphyte preparations.

Storage techniques are aimed at halting enzyme activity, which can alter adenine nucleotide composition. Enzyme activity may be minimized by either dehydration or freezing. Frozen-lyophilized-ground-desiccated tissue (Wilson, 1978) and frozen extract (Holm-Hansen, 1973) consititute two forms of storage. In this study, frozen-lyophilized-ground tissue was stored desiccated-dark for periods up to 5 days.

Extraction--Extraction of adenylates at *in situ* levels requires rapid nucleotide release and enzyme deactivation by either heating or lowering pH. Destruction of the semipermeable characteristics of cell membranes with boiling extractants causes all soluble constituents (e.g. adenylates) to rapidly diffuse out of the cells, ultimately resulting in a uniform concentration of each constituent throughout the entire suspension (Holm-Hansen, 1973). Hydrolases are released upon disruption of cellular integrity (DeGreef et al., 1979). Deactivation of these enzymes relies on the effectiveness of heat conduction or acid permeation through the tissue. The resultant thermal or $[H^+]$ gradients (Karl et al., 1978) are dependent on tissue chemical and physical properties (e.g. surface to volume ratio, density, chemical composition). Thermal gradients are minimized by homogenization of tissue and by using a low tissue to extractant ratio ($\leq 1\%$ w/v). Holm-Hansen and Karl (1978) recommend a sample to extractant ratio of $\leq 2\%$ (v/v).

Optimal extraction and recovery were achieved with boiling EDTA plus PVPP (pH 7.6). Boiling EDTA extraction of plant tissue has previously been proven effective (Mendelssohn and McKee, 1981; Guinn and Eidenbock, 1972). As a chelating agent, EDTA binds divalent metal cations which generally inhibit light output in the firefly bioluminescent reaction (Karl and LaRock, 1975). These authors caution against excessive EDTA addition which complexes Mg^{++}ions (required by luciferase), decreasing light output. Use of PVPP to adsorb phenols serves to increase light output. Higher plants contain phenolic compounds which bind proteins (Loomis and Battaile, 1966) and apparently inhibit luciferase (Mendelssohn et al., 1978). *Z. marina* is reported to contain several types of phenolic acids (Zapata and McMillan, 1979). Color quenching was also reduced in extracts treated with PVPP. Guinn and Eidenbock (1972) detected greater amounts of ATP in cotton leaves with polyvinylyrrolidone (PVP) treatment.

Recovery of internal standards does not assess extractant efficiency *per se*, since added adenylates are extracellular. However, internal standards are useful in evaluating apparent and/or real nucleotide losses due to hydrolysis, adsorption, coprecipitation, ionic interferences, turbidity, and color quenching (Karl, 1980).

A 30 sec extraction was selected for routine use, although no significant differences in adenylate levels were obtained from 5-120 sec. Mendelssohn and McKee (1981) found no significant difference with boiling EDTA plus PVPP extraction over 5-180 sec. However, Karl et al. (1978) caution against prolonged extraction which may hydrolyze nucleoside triphosphates. When using a boiling extractant, it is essential that the temperature be maintained at 100°C in order to deactivate ATPases (Holm-Hansen and Karl, 1978).

Leaf tissue extraction from either individual plants or a pooled plant sample masks adenylate variation on a cellular or organellar level. When multicellular tissue is extracted, mass-weighted mean adenylate values are determined. Cellular compartmentation and tissue heterogeneity may actually permit a range of co-existing metabolic states (Pradet and Raymond, 1978; Karl, 1980).

Conversion--TRICINE buffer (25 mM in firefly reaction) was selected for routine use, since this buffer yielded the highest light output. Webster et al. (1980) have also reported maximum light production with 25 mM TRICINE. Apparently, luciferase has the most favorable conformation in TRICINE.

Cofactor requirements have been specified for conversion enzymes, pyruvate kinase (PK) and adenylate kinase (AK), by Kayne (1973) and Noda (1973), respectively. Both PK and AK require a divalent cation (e.g. Mg^{++}). Without $MgSO_4$ addition, essentially no conversion of AMP or ADP occurs. Although the PK reaction also requires a monovalent cation (e.g. K^+), K_2SO_4 addition is not necessary. NH_4^+ (present in commercial PK and AK suspensions) and/or Na^+ (present in commercial EDTA and PEP salts) meet this requirement.

$MgSO_4$, K_2SO_4, and pH 8.1 quenched light output in the firefly reaction. DeLuca et al. (1979) report that $SO_4^=$ inhibits the reaction. Generally, cations and anions reduce light emission (Karl and LaRock, 1975). Apparently, sufficient Mg^{++} is contained in the FLE preparation to meet the luciferase divalent cation requirement (DeLuca, 1976). Additional $MgSO_4$ inhibits light output, but Mg^{++} is needed in conversion reactions. The pH optimum for the firefly reaction is in the range 7.4 (Strehler, 1968) to 7.8 (Webster and Leach, 1980). pH 7.6 was selected for routine use, since it falls within this range and yielded higher light output than pH 8.1.

The heat deactivation step is essential when using integral measurement. Heating denatures PK, preventing ATP production from reaction of PK and PEP with ADP contained in the crude FLE preparation. Karl and Holm-Hansen (1978) report that heat deactivation is not required when using peak height measurement with in situ AT >50 ng ml^{-1}, since PK interference is overwhelmed by the magnitude of the ATP-dependent peak light emission.

When ATP is <30 ng ml^{-1}, AMP conversion to ATP may be incomplete, since ATP is required to initiate the AK reaction (Karl and Holm-Hansen, 1978). An increase in ATP lowers the apparent K_m of AK for AMP. Since all sample extracts in this study contained >50 ng ml^{-1} ATP, addition of ATP was unnecessary.

Firefly lantern extract preparation--Reconstitution of lyophilized firefly lantern extract (FLE) with TRICINE buffer plus $MgSO_4$ (pH 7.6) was selected for routine use in order to stabilize pH. This procedure results in a final buffer concentration of 25 mM (firefly reaction mixture), the optimum prescribed by Webster et al. (1980). $MgSO_4$ addition complies with the recommendation by Karl and Holm-Hansen (1976) to add Mg^{++} when final FLE volume (25 ml) exceeds 5 ml, specified for Sigma FLE-50 by the manufacturer.

A 6-8 hr aging period at room temperature (Mendelssohn and McKee, 1981) was chosen as the routine procedure for FLE preparation. FLE was aged in order to degrade endogenous adenine nucleotides. Prolonged aging and high temperature result in loss of luciferin-luciferase activity. Karl and Holm-Hansen (1976) demonstrated that loss of Sigma FLE-50 activity over 36 hrs at 25°C was due to luciferin rather than luciferase degradation.

Although firefly luciferase is specific for ATP, transphosphorylases (e.g. NDPK) contained within crude luciferase preparations, regenerate ATP from other NTP's (DeLuca, 1976). Karl and Holm-Hansen (1978) reported that GDP addition to the FLE preparation (400 ng ml^{-1}) effectively inhibits ATP production from GTP, uridine triphosphate (UTP), inosine triphosphate (ITP), and cytidine triphosphate (CTP). Christensen and Devol (1980) observed no reduction in light emission with GDP addition.

In the present study, a greater amount of GDP (6.85 μg ml^{-1}) reduced light output in both standards and samples. Since standards contain no NTP (other than ATP), reduced light output with GDP addition reflects ATP consumption by mass-action adjustment via the NDPK reaction. Apparently, NDPK does not compete with luciferase for ATP (10 ng ml^{-1}) with GDP addition under 1 μg ml^{-1} (Karl and Nealson, 1980). Since sample adenylate levels showed no difference with or without GDP,GDP addition to the FLE preparation (6.85 g ml^{-1}) appears unnecessary.

Photometry--Since the time course of light production resulting from non-adenine NTP's is slower than *in situ* ATP-dependent light emission kinetics, interference is minimized with peak height measurement (Holm-Hansen and Karl, 1978). However, DeLuca et al. (1979) have stated that no single method of measuring light production is adequate for all conditions.

Parallel and linear log-log standard regressions between net light output and ATP (40-4000 ng ml^{-1}) were obtained with peak height (2 sec delay, 1 sec count) and integration (10 sec delay, 10 or 30 sec count). Webster and Leach (1980) demonstrated parallelism between peak height and integration (15 sec delay, 60 sec count) over 0.2-200 ng ml^{-1} ATP. A 10 sec delay, followed by a 10 sec integration, was selected as the routine counting method for two reasons: 1) mixing problems with peak height were avoided, and 2) after thorough mixing during a 10 sec delay, the shortest machine-available integral (10 sec) minimized time-dependent interferences.

Standard curve--Three standard curves, prepared with reagents for determination of [ATP] (Tube A), [ATP+ ADP] (Tube B), and [ATP + ADP + AMP] (Tube C), allow more accurate sample adenylate measurement than single curve determinations (Holm-Hansen and Karl, 1978). Use of multiple standard curves ensured ionic composition and ATP reactivity were similar in both standards and samples. All three log-log standard regressions between net light output and ATP (40-4000 ng ml^{-1}) were highly linear.

In this study, separate regressions were specifically required, due to $(NH_4)_2SO_4$ addition and heat deactivation. Quenching was lowest in Tube A and highest in Tube C. Commercial preparations of PK (Tubes B and C) and AK (Tube C) contain $(NH_4)_2SO_4$, which reduced light production. The heating step appeared to effectively denature PK but not AK. Selective PK deactivation causes the AK reaction to re-equilibrate with backproduction of ADP from ATP in solution (Tube C), reducing light emission (Karl and Holm-Hansen, 1978). Christensen and Devol (1980) reported a 15% reduction in peak height due to this re-equilibration.

Tissue Comparisons--

Zostera marina--Since leaves contain the highest adenylate levels among four tissues examined, it is suggested that leaf material be routinely sampled as the test tissue for adenylate analyses in *Z. marina*. Low adenylate levels in *Z. marina* root plus rhizome tissue are attributed to the presence of structural or metabolically inert material (Pamatmat and Skjoldal, 1979), as well as lowered aerobic respiration in reduced sediments (Mendelssohn et al., 1981). Tissue adenylate distribution in *Z. marina* contrasts with that observed for *Spartina alterniflora* (cordgrass), where leaf sheath and roots contained higher levels of ATP than leaves (Mendelssohn and McKee, 1981). This is presumably due to actively dividing meristematic tissue in leaf sheath and roots.

Tissue ATP level reflects ATP generation, utilization, and translocation. Light and oxygen availability permit both photo- and oxidative phosphorylation, respectively (Sellami, 1976), in aboveground tissue. Belowground tissue in reduced sediments must rely on limited oxidative phosphorylation, substrate phosphorylation in glycolysis (Mendelssohn et al., 1981), and possibly translocation (Thigpen, 1981) to maintain an adequate supply of ATP.

Mathematically, EC should be positively correlated with ATP and negatively correlated with AMP. AT should correlate positively with ATP, ADP, and AMP. All of these correlations were observed.

Other species--Although adenylate analytical techniques were specifically adapted to *Z. marina*, the methodology was applied to epiphytes of *Z. marina*, *Ruppia maritima* (a seagrass), and *Spartina alterniflora* (a marshgrass) for comparative purposes. As previously suggested, relatively low adenylate levels in epiphytic algae may result from metabolically inert material in epiphyte preparations. Adenylate content of *R. maritima* aboveground tissue was similar to that of *Z. marina* leaf tissue.

Differences in methodology and environment preclude strict comparison with the following values reported in the literature. *Thalassia testudinum*, a tropical seagrass, contained 703 ng ATP per leaf disc dry wt (485 μg ATP g^{-1} dry wt) one day after excision (Knauer and Ayers, 1977). This value represents about twice the amount observed for seagrasses (*Z. marina* and *R.*

maritima) in the present study. In a tissue study with *S. alterniflora*, Mendelssohn and McKee (1981) report a compartively high leaf concentration of 980 nmol ATP g^{-1} dry wt (495 μg ATP g^{-1} dry wt).

Seasonal Survey--

Although temperature, light, salinity, and nutrient regimes all exert an influence on growth (Setchell, 1929; Biebl and McRoy, 1971; Backman and Barilotti, 1976; Orth, 1977), temperature appears to be dominant in regulating the seasonal growth pattern of *Z. marina* in the Chesapeake Bay (Orth et al., 1981). In the present study, maximal shoot density and biomass occurred during spring. At a nearby site (inshore Guinea Marsh), peak shoot density and biomass were observed during June-July for the preceeding two years (Orth et al., 1981).

Aboveground tissue ATP levels were highest during winter and summer and lowest during spring and fall. Winter and summer correspond to periods of slow growth and senescence, respectively, with decreased rates of ATP utlization. In contrast, spring and fall correspond to periods of more rapid growth with increased rates of ATP utilization. Seasonal ATP levels in aboveground *Z. marina* contrasted with those reported for *Populus gelrica* (poplar) twigs, which contained greatest amounts of ATP during active growth and lowest amounts during the no growth season (Sagisaka, 1981).

Sexual reproduction in *Z. marina* occurs during spring in the Chesapeake Bay (Stevenson and Confer, 1978). This expenditure of energy may reduce ATP content. Low adenylate levels are also observed in *Corbicula fluminea* (freshwater clam) during periods of reproductive activity (Giesy and Dickson, 1981).

Belowground tissue ATP levels were highest during summer and fall and lowest during winter and spring. Belowground levels were generally much lower than corresponding aboveground levels. As previously suggested, low belowground adenylate levels may be attributed to metabolically inert material (Pamatmat and Skjoldal, 1979) or lowered aerobic respiration in reduced sediments (Mendelssohn et al., 1981).

Although amounts of adenine nucleotides are routinely reported, there is an important metabolic distinction between amount and turnover rate. The ATP turnover rate or energy flux through the adenine nucleotide pool is actually the more important quantitative assessment of cellular energetics (Weiler and Karl, 1979).

In both above and belowground *Z. marina* tissue, the following expected correlations were observed: 1) EC positively correlated with ATP and negatively correlated with AMP, and 2) AT correlated positively with ATP, ADP, and AMP. In aboveground tissue over the one year survey, ATP, ADP, and AMP comprised approximately 41-74%, 12-32%, and 7-31%, respectively, of the total adenylate pool. AT fluctuation demonstrates net synthesis and degradation of nucleotides.

Between month variability in EC was damped relative to individual adenylate concentrations. This was also observed in a seasonal study of adenine nucleotides in freshwater clams (Giesy and Dickson, 1981). Lower EC variability has both biochemical and mathematical rationales. EC is not only regulatory but is also regulated within narrow limits by enzymes, controlling rates of reactions which are coupled to the use and regeneration of ATP (Atkinson, 1977). It has been suggested that AMP removal by adenylate deaminase serves to buffer the cell against a sharp transient decrease in EC (Chapman and Atkinson, 1973). The presence of ATP and ADP in both numerator and denominator of the EC ratio further reduces variability.

Conclusions--

Due to the lability of adenine nucleotides, precautions must be taken throughout the analysis in order to quantify adenylates at their *in situ* levels. Freezing plants within 2 min after harvest, prevention of thawing, and lyophilization minimized adenylate change. Prolonged desiccated or frozen storage should be avoided, and hydrated extracts must be held on ice during the assay. High recovery rates of internal standards, added immediately prior to extraction, indicated minimal adenylate loss after extraction during the remainder of the assay. An additional methodological step is unique to aquatic macrophytes. *Z. marina* leaves should be scraped free of epiphytic algae after lyophilization, since substantial epiphytic biomass obscures leaf nucleotide content.

The tissue comparison and seasonal survey provide baseline information on natural adenylate variability in *Z. marina*. Since leaf tissue contained the highest adenylate levels, leaves appear most suitable as a test tissue for routine adenylate analyses. Seasonal ATP levels in aboveground tissue reflect energy expenditures associated with growth patterns.

The method presented for the determination of adenine nucleotides in *Z. marina* has several limitations. Tissue adenylate measurement results in a mass-weighted mean value and provides no information on intercellular heterogeneity or intracellular compartmentation. Adenylate levels determined in metabolic or environmental studies with this technique should be interpreted in this context. Direct application of this methodology to other species may be inappropriate. With slight modification, however, the technique should prove suitable to other important macrophyte species.

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ATRAZINE EXPERIMENTS

Introduction

The role of adenine nucleotides in cellular bioenergetics, including adenylate energy charge (EC) theory, has been presented by Atkinson (1977). Adenine nucleotides are strategically adapted to metabolic regulation, since they are operationally linked with nearly all metabolic sequences. The EC ratio, [ATP] + 1/2 [ADP]/([ATP]+[ADP]+[AMP]), represents a linear measure of the metabolic energy stored in the adenylate pool, ranging from 0 (all AMP) to 1.0 (all ATP). EC regulates metabolic sequences by controlling enzymic rates of reactions which are coupled to the use and regeneration of ATP.

Since the metabolic energy state of an organism is sensitive to environmental variation, EC has been proposed as an index of sublethal stress (Ivanovici, 1980) and has been widely applied in this context (e.g. Romano and Daumas, 1981; Giesy et al., 1981; Mendelssohn and McKee, 1981). The present study evaluates effects of herbicide on adenylate response patterns in Zostera marina (eelgrass), a submerged marine angiosperm. Z. marina is an ecologically important mactrophyte species (McRoy and Helfferich, 1977; Stevenson and Confer, 1978; Phillips and McRoy, 1980; Wetzel et al., 1981; Orth et al., 1981), occurring in temperate and subarctic coastal and estuarine waters in the Northern Hemisphere (den Hartog, 1970).

Atrazine, a triazine herbicide, is widely used for selective control of broadleaf and grassy weeds in tolerant crop species, including corn, sorghum, and sugarcane (WSSA, 1974). As an inhibitor of the Hill reaction in photosynthesis (Ebert and Dumford, 1976; Gardner, 1981), atrazine is expected to impair photoevolution of oxygen, net photoreduction, and noncyclic photophosphorylation in the chloroplast and may adversely affect the adenylate pool. Several factors, which may eliminate or offset atrazine toxicity, are reduced herbicide uptake and translocation (Ebert and Dumford, 1976), detoxication (Shimabukuro et al., 1971), or compensatory phosphorylation potential. Since neither cyclic photophosphorylation (Thompson et al., 1974) nor oxidative phosphorylation (Davis, 1968) are appreciably altered by atrazine, these processes along with substate phosphorylation may regenerate adequate amounts of ATP.

Several studies have investigated ATP response to atrazine exposure in higher plants. Atrazine, administered through leaves (500 ppm) or through roots (0.5 ppm), generally decreased ATP content in Cucumis sativus (cucumber) leaves and roots over 1-3 days (Decleire and Decat, 1981). In contrast, Gruenhagen and Moreland (1971) have reported slightly elevated levels of ATP in Glycine max (soybean) hypocotyls with atrazine exposure (43 ppm) over 6 hrs. These inconsistent results may reflect differences in exposure time or differences between species in atrazine metabolism or phosphorylation potential.

Nontarget effects of atrazine have been implicated in recent declines of submerged aquatic macrophytes in the Chesapeake Bay (Stevenson and Confer, 1978). Agricultural runoff, leaching, and aerial transport processes introduce atrazine into the Bay (Wu, 1981). Forney and Davis (1981) have reported 3-6 week I_1 values (the concentration inhibiting growth 1%) of a few ppb atrazine for several submerged aquatic macrophyte species. Although atrazine levels in the Chesapeake Bay are generally below 1 ppb (Correll et al., 1978; Wu et al., 1980; this study, Section 3), results presented in Section 4 suggest that *Z. marina* beds in the lower Bay may experience atrazine concentrations, ranging from 1-10 ppb, for several days over the growing season.

Assuming *Z. marina* is susceptible to atrazine toxicity, decreased ATP and EC levels with atrazine exposure are expected. This study investigates adenylate response patterns in *Z. marina* over short-term (6 hr) and long-term (21 day) atrazine exposure. Production, morphometric, and mortality data were collected in order to facilitate interpretation of adenylate response to atrazine.

Methods

Field Collection and Transplanting--

Location of the *Zostera marina* sampling site in the lower Chesapeake Bay is described in Method Development of this chapter. Clumps of eelgrass were uprooted with a shovel, swirled in river water to remove macro-algae and loose sediment, transported in a bucket of river water to the laboratory, and acclimated in a flow-through system. Clumps were then divided into "individual" plants (i.e. single shoot with the attached leaf cluster and a 2-5 cm rhizome segment) for transplanting. Transplants were planted in natural sediment (obtained from the VIMS beach) in Jiffy Pots. All transplants were submerged in a flow-through system.

Adenine Nucleotides--

Samples were processed, as described in Method Development Section of this chapter, with the following specifications:

1) transplants were uprooted by hand,
2) for each treatment, plants were pooled in order to minimize within treatment variation and spotlight between treatment variation,
3) leaf tissue was assayed at the end of short-term (6 hr) and longterm (21 day) atrazine experiments, and
4) photometry was performed entirely in the integration mode.

Environmental Data--

Environmental data included water temperature, salinity, dissolved oxygen (DO), and photosynthetically active radiation (PAR). Minimum and maximum temperatures were recorded with a min-max thermometer (Taylor Instruments). Salinity was measured with an induction salinometer (Model RS 7B, Beckman). DO was monitored polarographically (Hitchman, 1978) with an oxygen meter (Model 2604, Orbisphere Corp.). This meter was calibrated in water-saturated air at specified temperature and pressure. Because it was not salinity-correctred, DO values are relative and not absolute. PAR was measured with a light meter (Model LI-185B, Lambda Instruments Corp.), equipped with a quantum sensor (Model LI-1905, Lambda).

Short-Term (6 Hr) atrazine Experiments--

Design--Effects of atrazine exposure over 6 hrs were tested in two sealed 37 l glass tanks, one control and one dosed chamber. The flow-through system inside a greenhouse is diagrammed in Figure 7.17. Nominal atrazine concentrations of 10 and 100 ppb were evaluated in two separate experiments. Design specifications are presented in Table 7.43.

Atrazine stock solution was prepared with technical grade atrazine (97.2%, CIBA-GEIGY Corp.), dissolved in glass-distilled methanol (Burdick and Jackson Labs). This solution was metered in with a peristaltic pump (Model 600-1200, Harvard Apparatus Co., Inc.), so that dilution yielded the desired atrazine concentration (0.07% v/v methanol). Flow rates were checked hourly. Short-term experiments did not incorporate a methanol control.

Atrazine--Water samples were collected, filtered, extracted, and assayed for atrazine by gas chromatography, as described in Section II. The gas chromatograph (Model 560, Tracor) was equipped with a nitrogen-phosphorus detector (Model 702, Tracor). Samples were collected to spot-check nominal atrazine concentrations.

Productivity--*Z. marina* productivity measurements are obtained, using the flow through system. Water was pumped through a 1   cartridge filter, as shown in Figure 7.17. Potted plants were placed in tanks, which were tightly sealed with glass tops, leaving no air space. After the tank water had turned over one time, DO was monitored hourly at both inflow and outflow ports. Dry weight of aboveground biomass in each tank was obtained at the end of the experiment.

Productivity was calculated from the following formula:

$$\text{mg } O_2 \text{ g}^{-1} \text{ hr}^{-1} = (\ \text{mg } O_2 \text{ l}^{-1})\ (\text{l tank})\ (\text{g dry wt})^{-1}\ (\text{hr turnover})^{-1}$$

where Δ = outflow DO - inflow DO

The ratio, tank volume/turnover time, is simply the flow rate. These production rates represent net productivity, since photosynthesis and respiration operate simultaneously during daylight hours.

Long-Term (21 Day) Atrazine Experiments--

Design--Effects of atrazine exposure over 21 days were tested in six pairs (each pair consists of A and B replicates) of 38 l glass tanks, corresponding to the following nominal atrazine concentrations: 0, 0.1, 1.0, 10, 100, 1000 ppb. Each tank initially held 15 potted plants. The flow-through system inside a greenhouse is diagrammed in Figure 7.18. Mean tank turnover times ranged from 7.3-13.5 hrs. This experiment was replicated four times. Replicate Experiments 1-4 were analyzed separately, as well as together, in some cases. Spot-check atrazine measurements are listed in Table 7.44.

Atrazine stock solutions were metered in with a peristaltic pump, so that dilution yielded the desired atrazine concentrations (0.07% v/v

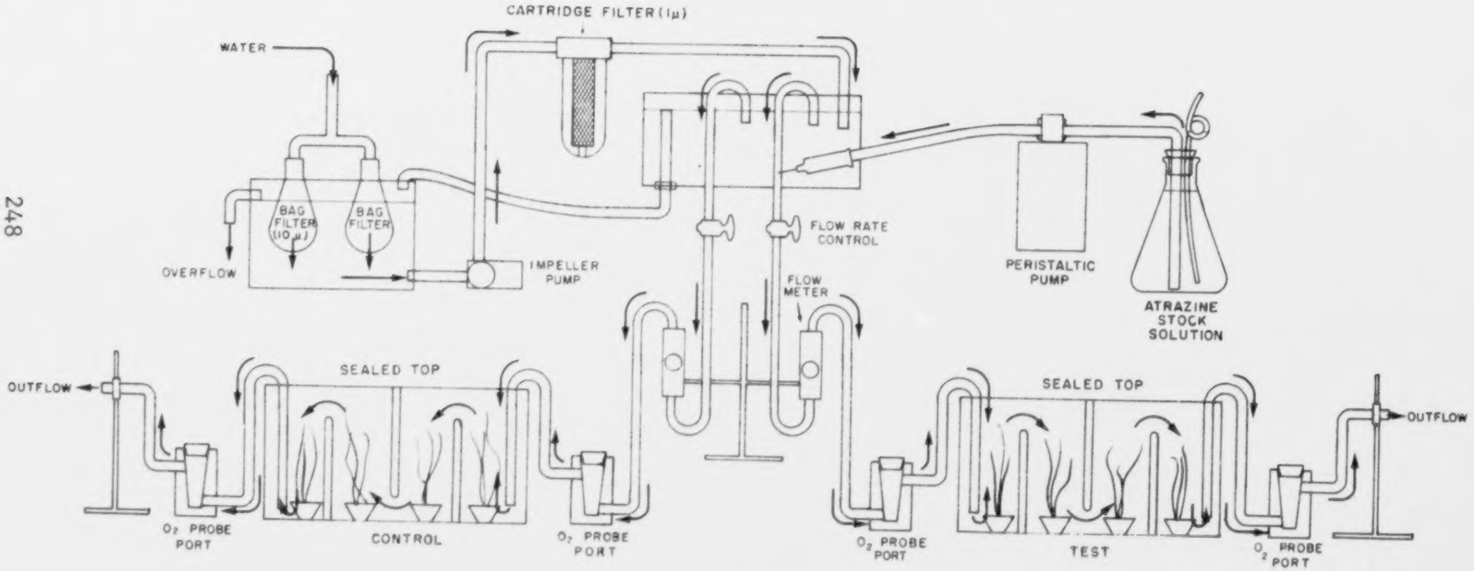
WATER
CARTRIDGE FILTER (1μ)
BAG FILTER (10 μ)
BAG FILTER
OVERFLOW
IMPELLER PUMP
FLOW RATE CONTROL
FLOW METER
PERISTALTIC PUMP
ATRAZINE STOCK SOLUTION
OUTFLOW
SEALED TOP
O2 PROBE PORT
CONTROL
O2 PROBE PORT
O2 PROBE PORT
SEALED TOP
TEST
O2 PROBE PORT
OUTFLOW

Figure 7.17. Flow-through system used short-term (6 hour) atrazine experiments.

TABLE 7.43. DESIGN SPECIFICATIONS FOR SHORT-TERM (6 HOUR) ATRAZINE EXPERIMENTS

Specification		Nominal Atrazine (ppb) 10	 100
Measured atrazine (ppb):	Initial	15.77	97.86
	Final	9.39	91.33
Exposure period (hrs)		1000-1630	1030-1700
Tank turnover time (hrs)		1.74	1.74
Aboveground dry wt (g):	Control	15.04	9.23
	Test	12.48	9.51

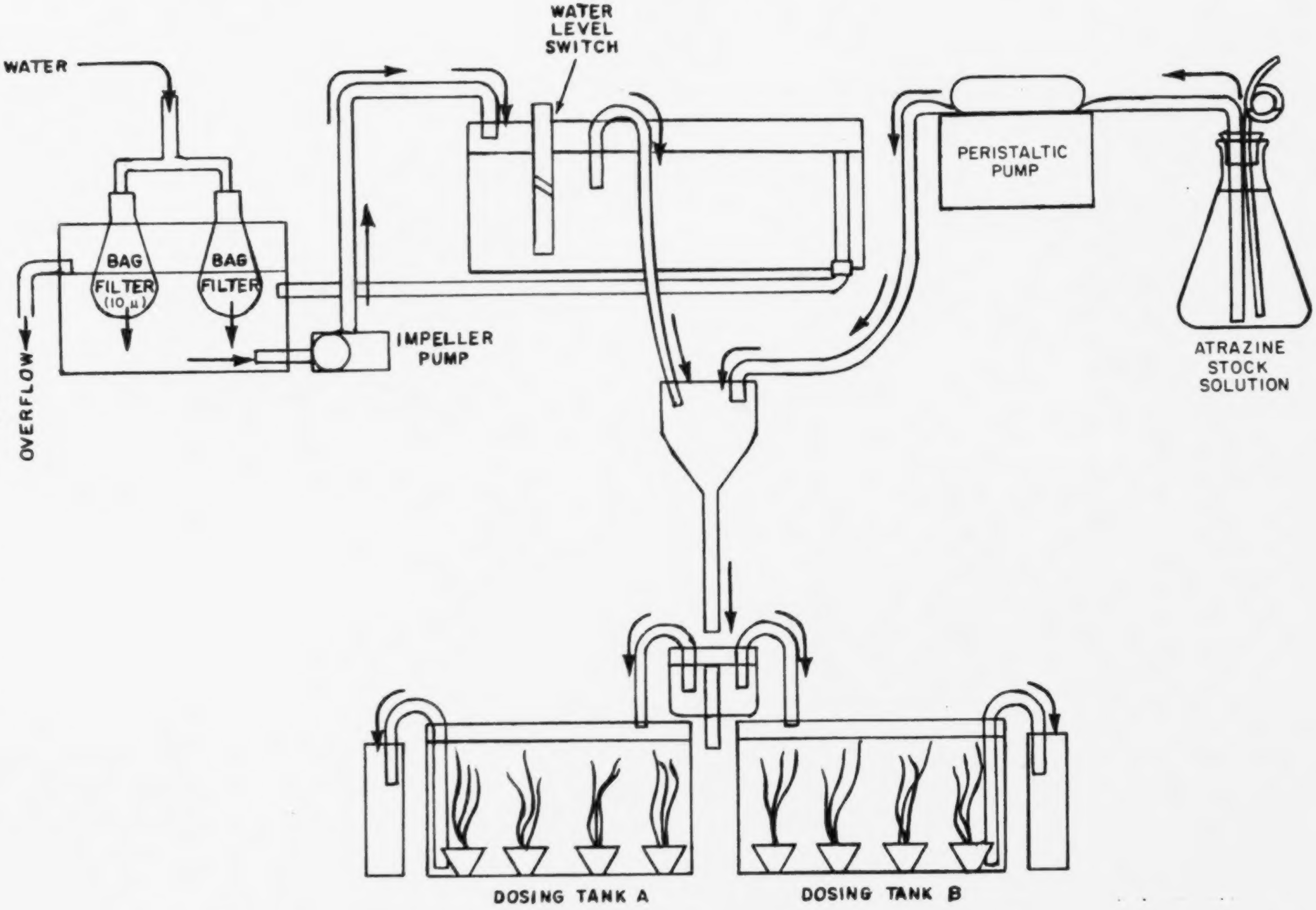
WATER
OVERFLOW
BAG FILTER (10 µ)
BAG FILTER
IMPELLER PUMP
WATER LEVEL SWITCH
PERISTALTIC PUMP
ATRAZINE STOCK SOLUTION
DOSING TANK A
DOSING TANK B

Figure 7.18. Flow-through system used for long-term (21 day) atrazine experiments. The diagram depicts only one of the six pairs of tanks in the system.

Table 7.44. SPOT-CHECK ATRAZINE MEASUREMENTS IN LONG-TERM (21 DAY) ATRAZINE EXPERIMENTS

Experiment	Exposure Time (days)	Atrazine (ppb)	
		Nominal	Measured
1	21	100	108.60
2	7	1	2.91
		10	22.49
		100	113.53
		1000	1051.08
	14	1	1.06
		1000	1038.69
	21	1	1.26
		1000	1072.86
3	21	.1	.70
		10	11.12
4	21	1	1.27
		100	116.09

methanol). Flow rates were monitored daily. Long-term experiments incorporated a methanol control.

Atrazine--Atrazine measurement was performed according to the procedure described for short-term experiments.

Morphometrics--Shoot length and number of leaves were obtained on all living plants from each tank at 0, 7, 14, and 21 days. Measurements from replicate tanks A and B were combined to calculate means. Weekly morphometric changes were calculated from the following formula:

$$\% \text{ Change} = \frac{\bar{X}_t - \bar{X}_o}{\bar{X}_o} \times 100$$

where $\bar{X}_t$ = mean at time t

$\bar{X}_0$ = mean at time zero

Mortality--Mortality was recorded in each tank at 7, 14, and 21 days. Dead plants were removed from the system. Visual criteria for plant death were loss of green pigmentation (i.e. chlorophyll degradation) and loss of structural integrity. Mortality observations from replicate tanks A and B were combined in tabulations.

Statistical Analysis--

The following procedures in the SPSS software package (Nie et al., 1975; Hull and Nie, 1981) were used: ONEWAY (single factor ANOVA, Hartley Fmax test for homoscedasticity, Student-Newman-Keuls multiple range test), SCATTERGRAM (linear regression and Pearson correlation), NPAR TESTS (Kruskal-Wallis single factor ANOVA by ranks and Kolmogorov-Smirnov one sample test for normality), and NONPAR CORR (Spearman rank correlation).

Other statistical procedures employed included nonparametric multiple range testing by rank sums (Zar, 1974) and dose-effect analysis with log-probit transformation (Litchfield and Wilcoxon, 1949).

Short-Term (6 Hr) Atrazine Experiments--Differences between adenylates, resulting from exposure to atrazine, were detected by the procedure diagrammed in Figure 7.19.

Long-Term (21 Day) Atrazine Experiments--Differences between adenylates, resulting from exposure to atrazine, were detected and located by the procedure diagrammed in Figure 7.19. Morphometric change was regressed against time for a control and five atrazine concentrations. Relationships between adenylate and atrazine data were evaluated by Spearman rank correlation. Median and 1% lethal atrazine concentrations (LC 50 and LC 1, respectively) and slope function (S), together with their 95% confidence limits, were estimated by log-probit analysis. Differences between these mortality statistics from replicate experiments were evaluated.

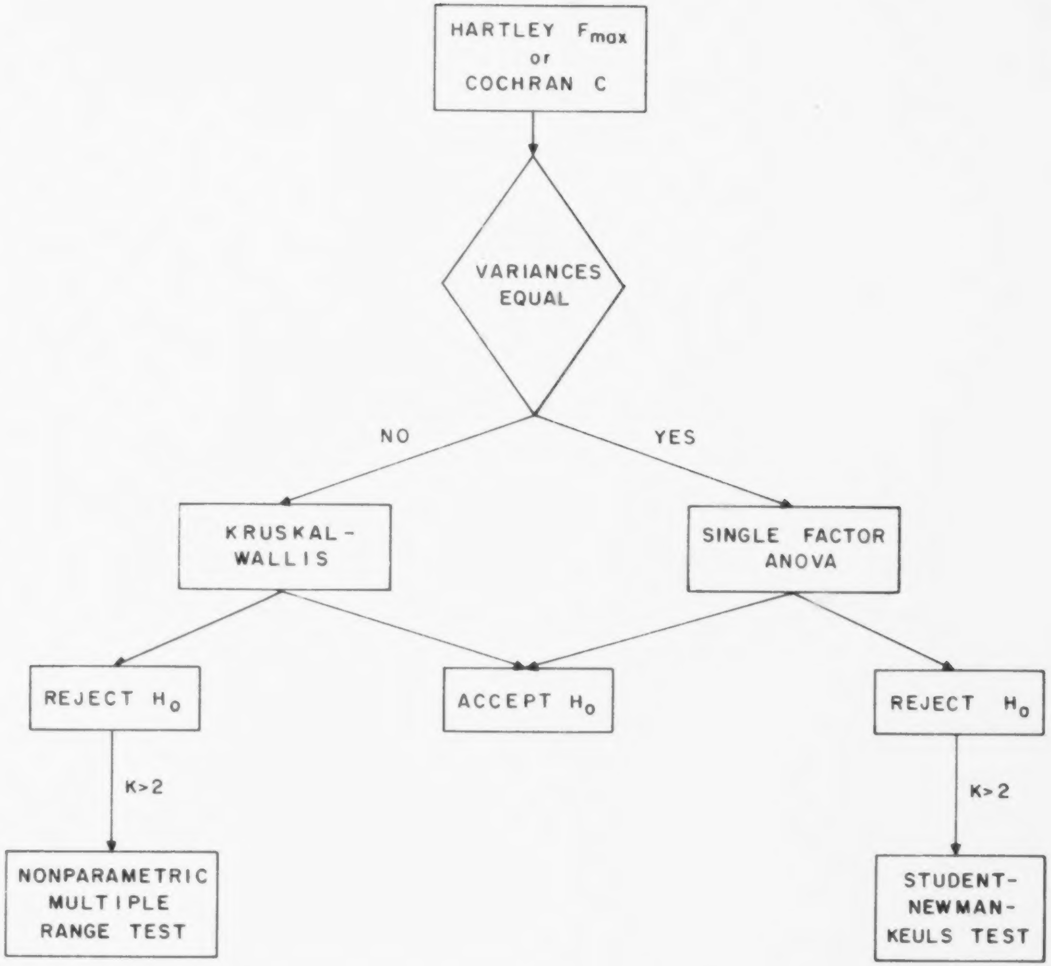
HARTLEY F_{max}
or
COCHRAN C
VARIANCES EQUAL
NO
YES
KRUSKAL-WALLIS
SINGLE FACTOR ANOVA
REJECT H_o
ACCEPT H_o
REJECT H_o
K>2
K>2
NONPARAMETRIC MULTIPLE RANGE TEST
STUDENT-NEWMAN-KEULS TEST

Figure 7.19. Detection and location of treatment differences.

Results

Short-Term (6 Hr) Atrazine Experiments--

Flow-through system data--Mean temperature and salinity are presented in Table 7.45. Similar and stable temperature and salinity prevailed over the course of the two experiments.

Productivity--Hourly net production rates in both control and test tanks, along with surface PAR readings, are plotted in Figures 7.20 and 7.21 for 10 and 100 ppb atrazine experiments, respectively. At 10 ppb atrazine, net productivity was positive and relatively similar in both control and test tanks. At 100 ppb atrazine, net productivity was positive in the control but generally negative in the test tank. These results indicate that 10 ppb atrazine had little effect on net productivity over 6 hrs, whereas 100 ppb exerted a marked negative effect.

Adenine Nucleotides--Adenylate and EC values in both control and test tanks are shown in Figures 7.22 and 7.23 for 10 and 100 ppb atrazine experiments, respectively. Results at both 10 and 100 ppb were the same. EC values in control and test tanks show no significant difference, whereas ATP, ADP, AMP and AT in test tanks were all significantly lower than their controls at both 10 and 100 ppb atrazine over 6 hrs.

Long-Term (21 Day) Atrazine Experiments--

Flow-through system data--Mean temperature and salinity, in four replicate experiments, are presented in Table 7.46. Mean minimum and maximum temperatures in Experiment 1 were considerably lower than corresponding temperatures in Experiments 2-4. Salinity was similar in all replicate experiments.

Morphometrics--Mean shoot length and number of leaves, obtained at the start of each experiment, appear in Table 7.47. Mean changes in shoot length and leaf number at 7, 14, and 21 days, for each atrazine concentration, were pooled from replicate experiments and regressed against time (Figures 7.24 and 7.25, respectively). Statistics associated with these regressions are presented in Table 7.48. Negative slopes and correlation coefficients for shoot length change at 1000 ppb atrazine and for leaf number change at both 100 and 1000 ppb have clearly demonstrated a negative effect of atrazine on growth over 21 days.

Mortality--Twenty-one day mortality, expressed as percent dead, is presented in Table 7.49 for replicate Experiments 1-4. Mortality in controls was <7%, which is acceptable in acute bioassays (Sprague, 1973). Mortality was 100% at 1000 ppb atrazine over 21 days in all replicates, with the exception of Experiment 1.

Results, derived from log-probit analysis of 21 day mortality data, appear in Table 7.50. Estimates of mortality statistics in Experiments 3 and 4 were very similar. The relatively large slope function, (S) in Experiment 1, due to incomplete mortality at 1000 ppb, was significantly higher than that obtained in either Experiment 3 or 4 and was reflected in the wide confidence limits, associated with LC 1 and LC 50 values in Experiment 1. The LC 50

TABLE 7.45. TEMPERATURE AND SALINITY DURING SHORT-TERM (6 HOUR) ATRAZINE EXPERIMENTS

Nominal Atrazine (ppb)	Temperature (°C) (n=52)		Salinity (°/oo) (n=1)
	$\bar{x}$	SE	
10	20.5	.1	21.97
100	22.5	.2	22.56

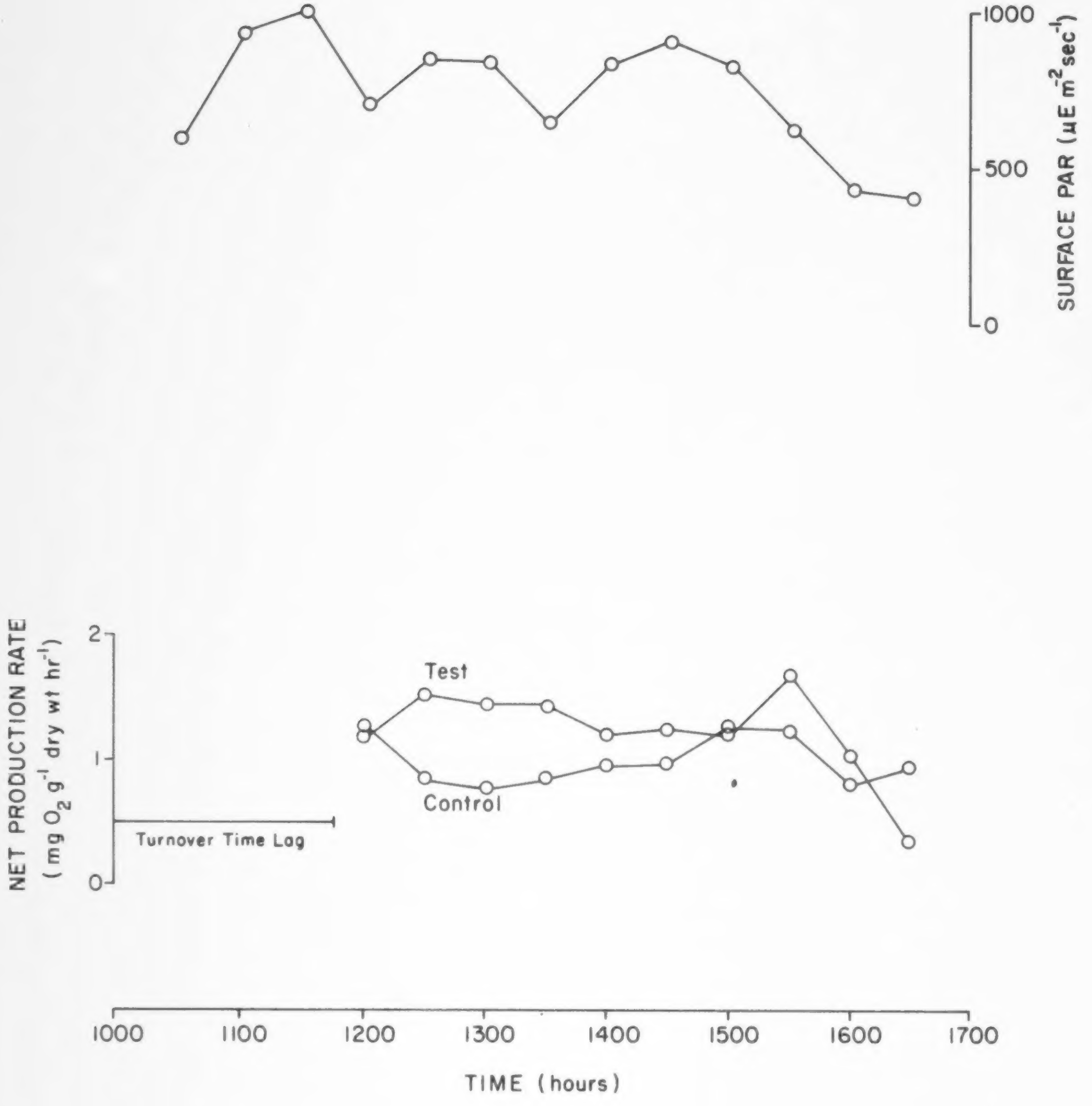

Figure 7.20. Surface PAR and net production rates during short-term (6 hour) 10 ppb atrazine experiment. Tank sealed at 1000 hrs.

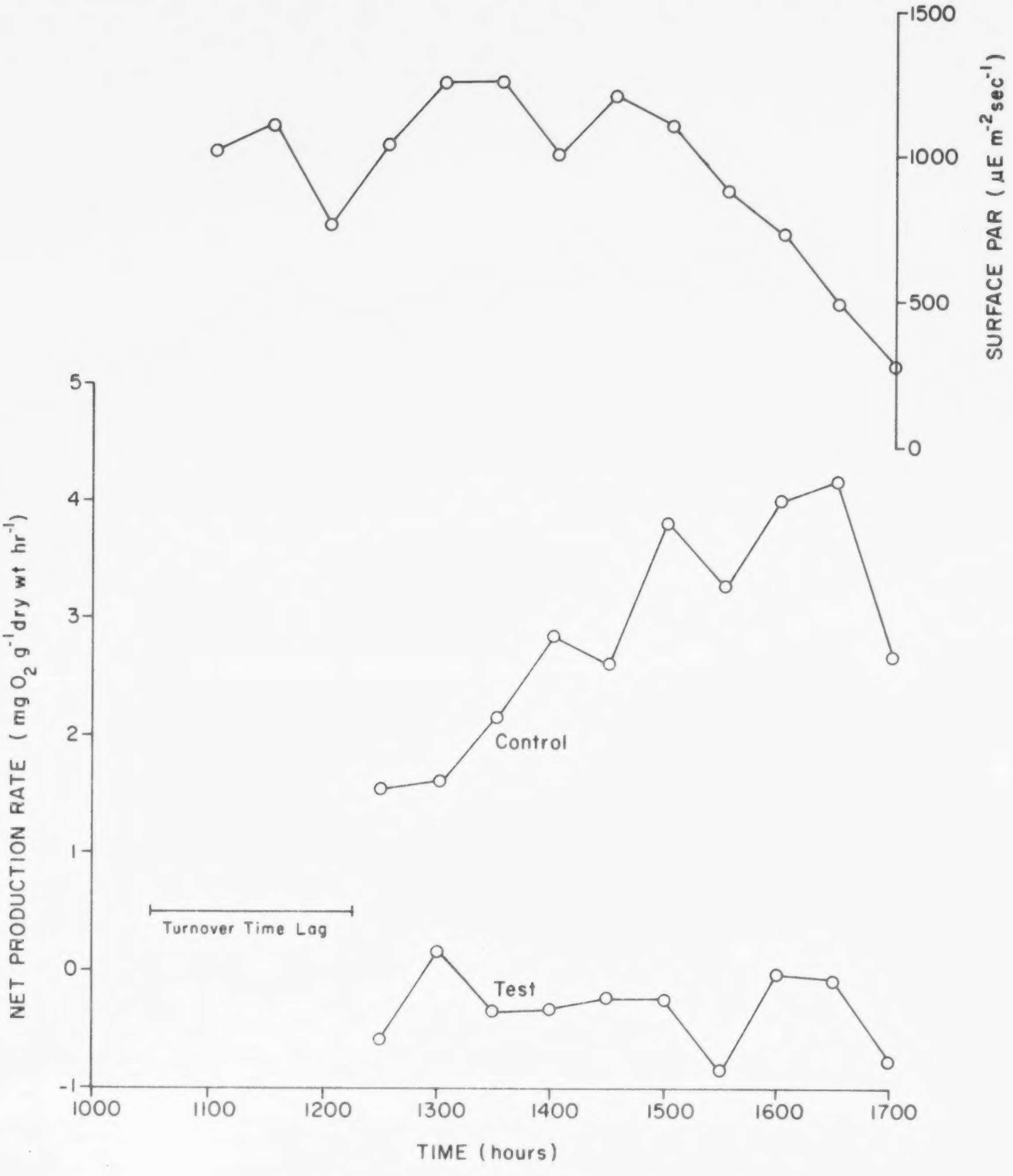

Figure 7.21. Surface PAR and net production rates during short-term (6 hour) 100 ppb atrazine experiment. Tank sealed at 1030 hrs.

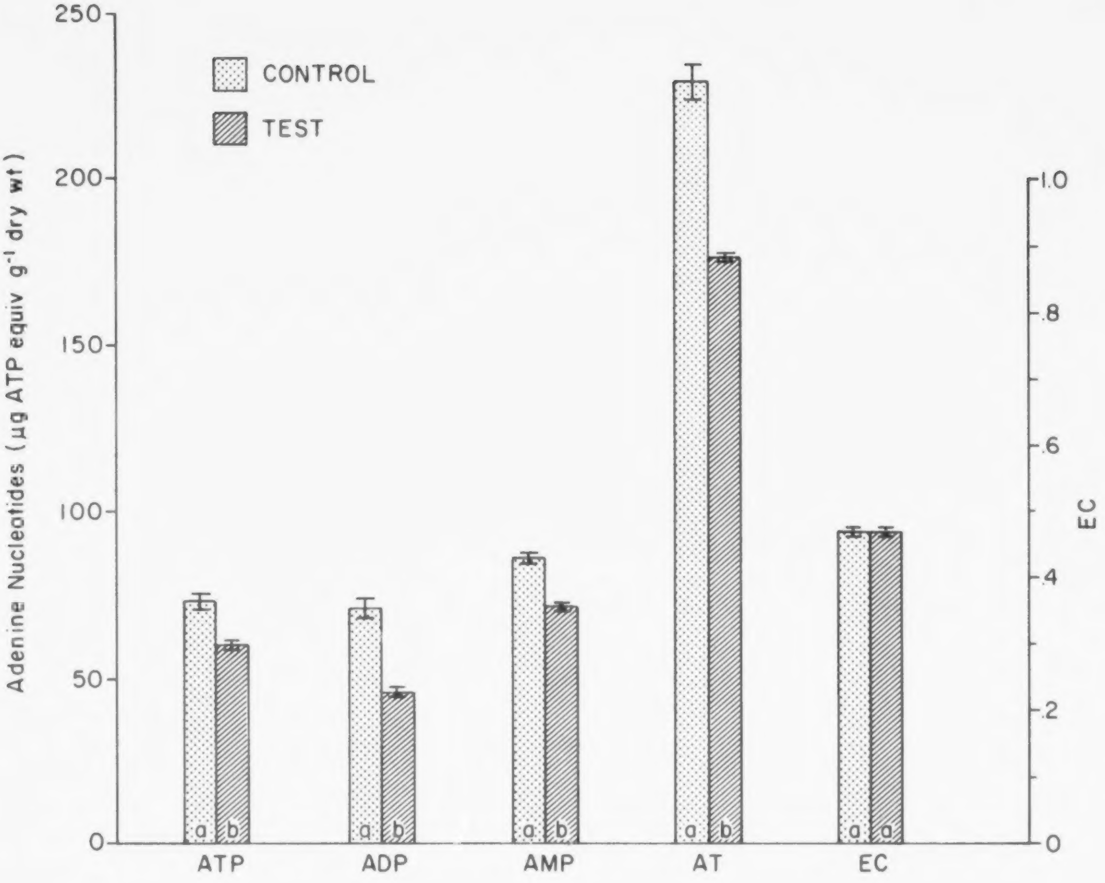

Figure 7.22. Adenine nucleotides and EC after 6 hours in the short-term 10 ppb atrazine experiment (n =4). Control-test pairs with same letters do not differ significantly (P >.05). Error bars are 1 standard error.

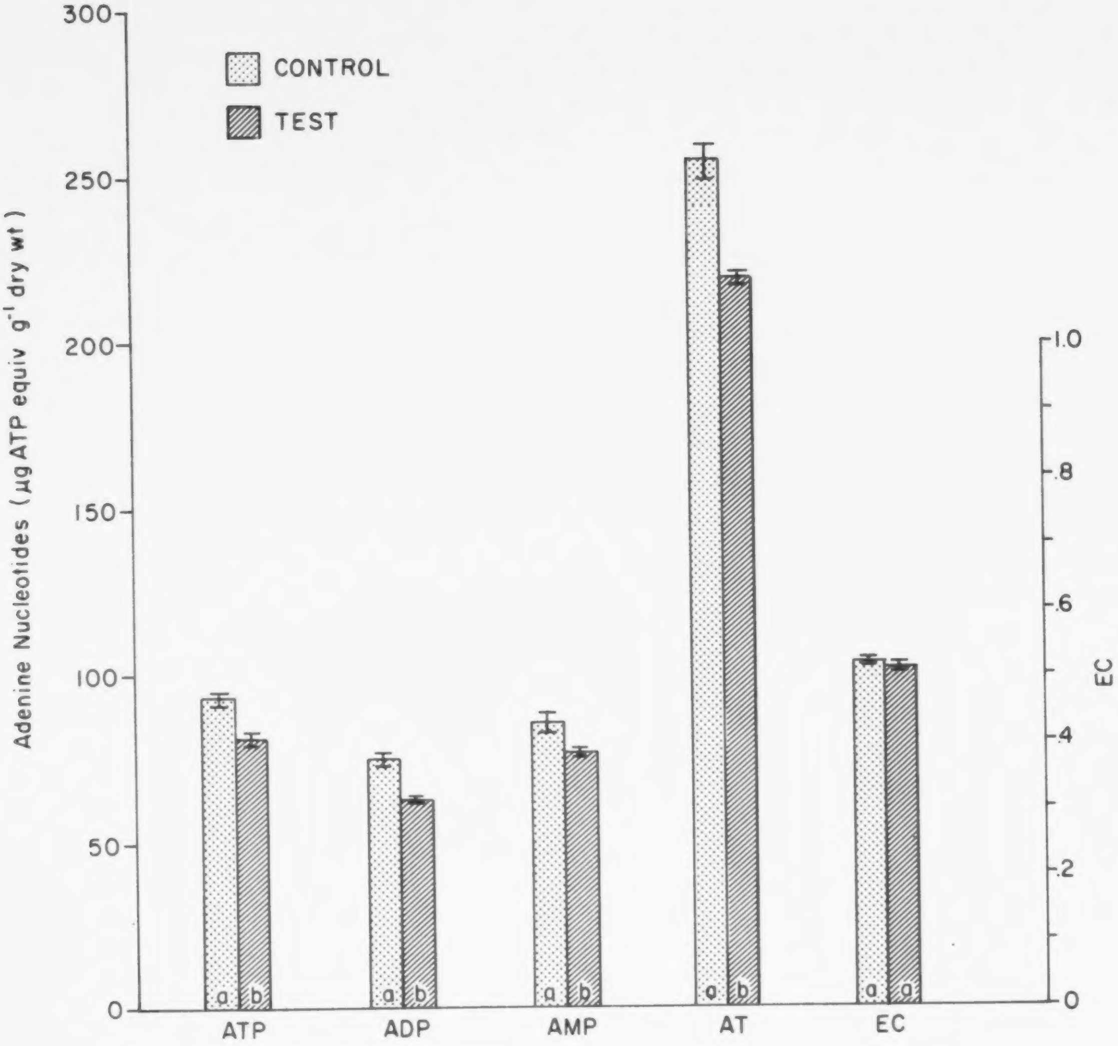

Figure 7.23. Adenine nucleotides and EC after 6 hours in the short-term 100 ppb atrazine experiment (n = 4). Control-test pairs with same letters do not differ significantly ($P > .05$). Error bars are 1 standard error.

TABLE 7.46. TEMPERATURE AND SALINITY DURING LONG-TERM (21 DAY) ATRAZINE EXPERIMENTS

Experiment	Temperature (°C)					Salinity (°/oo) (n=1)
	n	Minimum		Maximum		
		$\bar{x}$	SE	$\bar{x}$	SE	
1	9	6.3	.5	16.0	.9	21.96
2	9	13.4	.9	25.3	.5	20.14
3	14	15.2	.6	24.6	.6	20.14
4	9	18.7	.7	28.4	1.3	19.13

TABLE 7.47. INITIAL *Z. MARINA* MORPHOMETRICS IN LONG-TERM (21 DAY) ATRAZINE EXPERIMENTS

Experiment	n	Shoot Length (cm)		Number Leaves	
		$\bar{x}$	SE	$\bar{x}$	SE
1	180	12.9	.3	3.4	.1
2	165	13.2	.3	4.2	.1
3	180	20.0	.4	4.5	.1
4	180	31.0	.8	5.3	.2

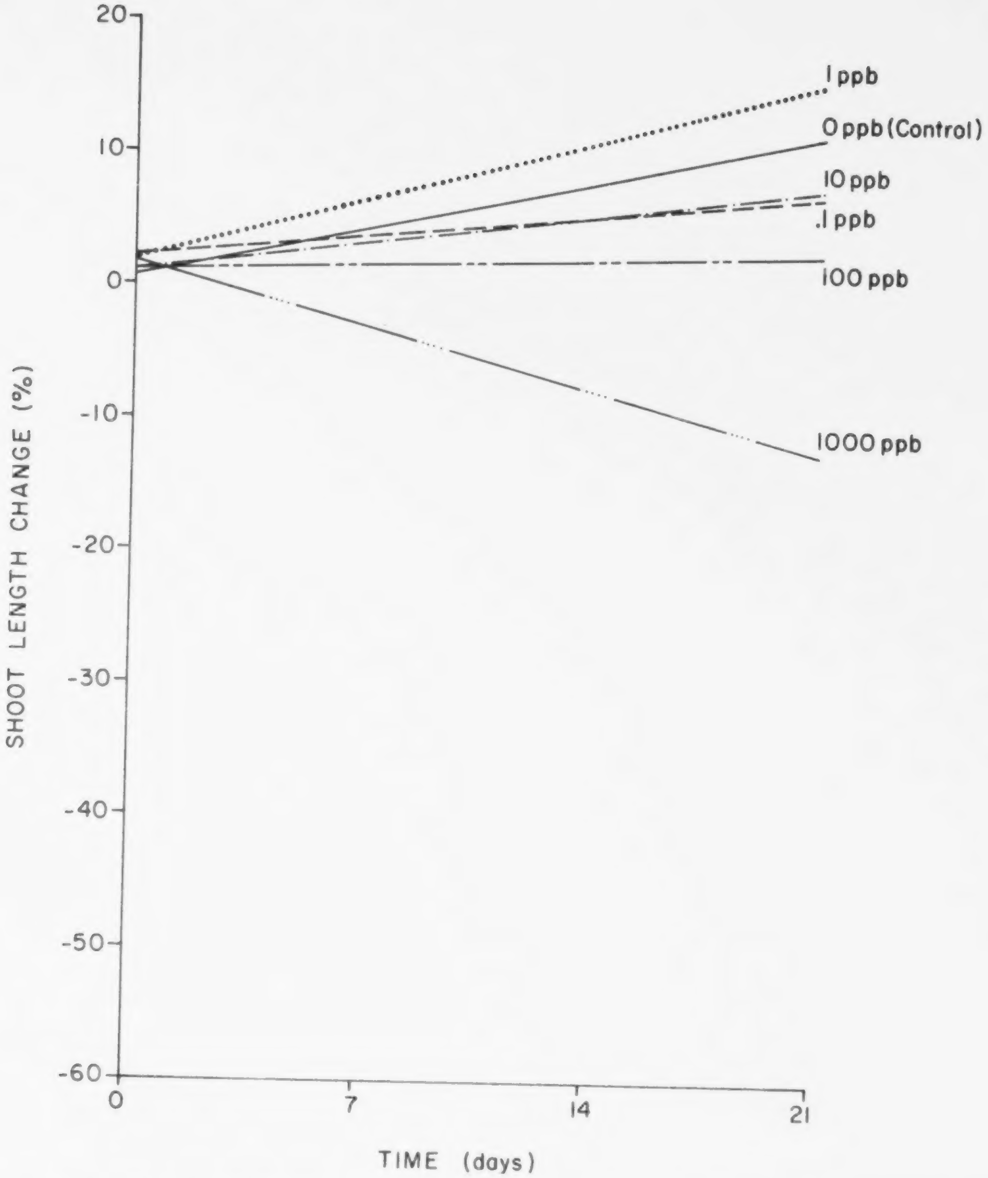

Figure 7.24. Regressions of shoot length change vs. time for control and five atrazine concentrations in the long-term (21 day) atrazine experiments. Data from replicate experiments are pooled.

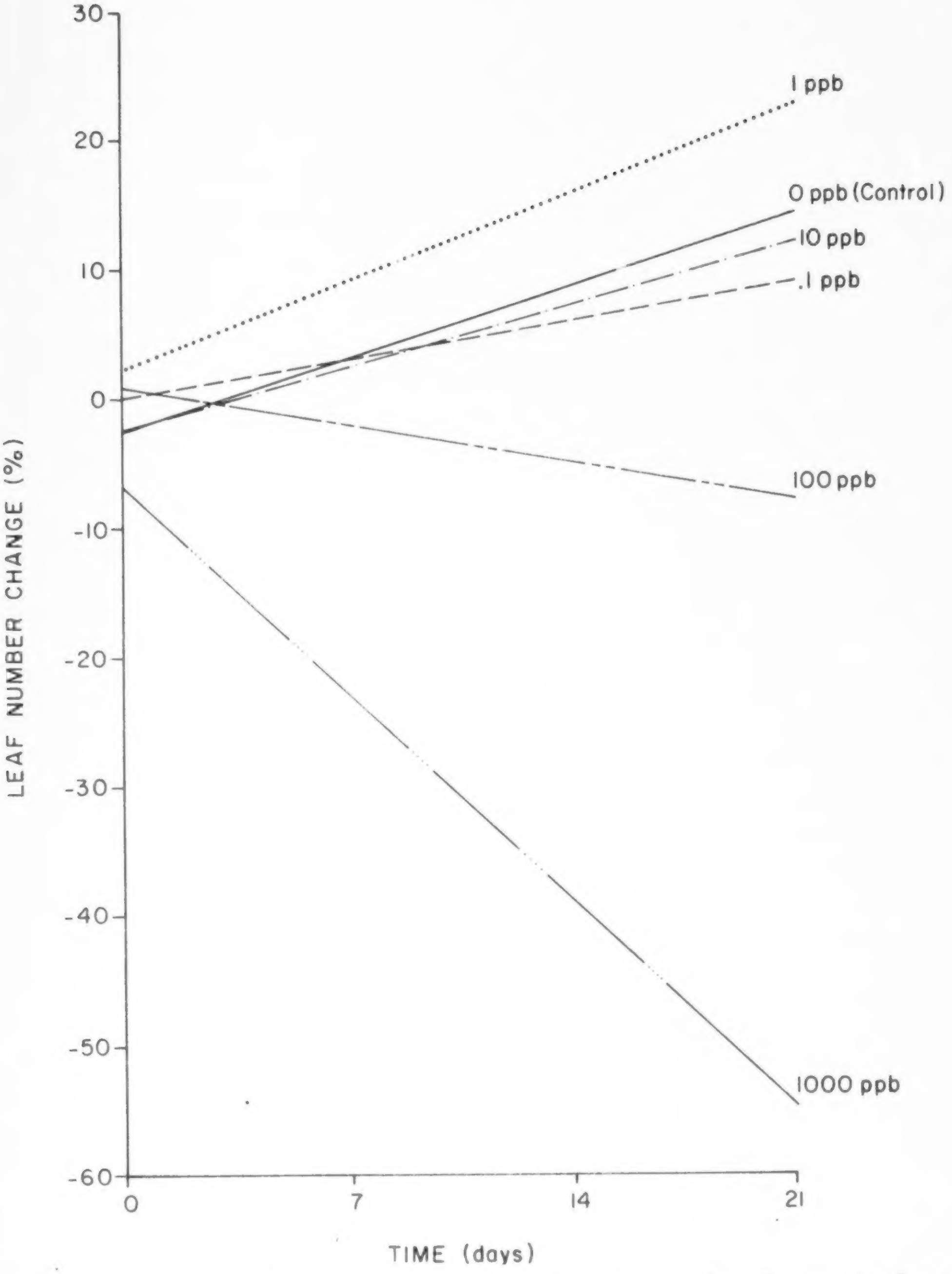

Figure 7.25. Regessions of leaf number change vs. time for control and five atrazine concentrations in the long-term (21 day) atrazine experiments. Data from replicate experiments are pooled.

TABLE 7.48. SHOOT LENGTH AND LEAF NUMBER CHANGE REGRESSION STATISTICS IN THE LONG-TERM (21 DAY) ATRAZINE EXPERIMENTS. DATA FROM REPLICATE EXPERIMENTS ARE POOLED

Nominal Atrazine (ppb)	n	Shoot Length			Number Leaves		
		Slope	Intercept	Correlation Coefficient	Slope	Intercept	Correlation Coefficient
0	16	.5354	.5600	.7100*	.8250	-2.7750	.4017
.1	16	.2318	2.1600	.2403	.4325	.0900	.4142
1	16	.6396	2.0900	.6181*	.9757	2.4675	.5574*
10	16	.3121	1.1225	.3947	.6954	-2.2450	.4886
100	16	.0718	1.2775	.1385	-.4161	.9500	-.2673
1000	11	-.6671	1.7700	-.4811	-2.2671	-7.1119	-.6047*

* $P < .05$

TABLE 7.49. MORTALITY (%) AFTER 21 DAYS IN THE LONG-TERM ATRAZINE EXPERIMENTS (N_O=30)

Experiment	Nominal Atrazine (ppb)					
	0	.1	1.0	10	100	1000
1	6.7	13.3	10.0	10.0	20.0	50.0
2	3.3	0[1]	10.0	10.0	46.7	100.0
3	3.3	0	0	0	3.3	100.0
4	6.7	3.3	3.3	0	10.0	100.0

[1] n_o = 15

TABLE 7.50. TWENTY-ONE DAY 1% (LC 1) AND 50% (LC 50) LETHAL CONCENTRATION, ALONG WITH SLOPE FUNCTION (S), IN THE LONG-TERM ATRAZINE EXPERIMENTS. LC 1, LC 50, AND ASSOCIATED CONFIDENCE LIMITS (CL) ARE EXPRESSED AS PPB ATRAZINE. S AND ASSOCIATED CL ARE UNITLESS

Experiment	LC 1	LC 1 95% CL	LC 50	LC 50 95% CL	S[1]	S 95% CL
1	1.9^{ab}[2]	.1-35.0	540^{a}	229-1274	11.02^{a}	3.37-36.03
2	2.6^{b}	.4-16.4	100^{b}	45-221	4.78^{ab}	2.35-9.70
3	38.7^{a}	16.5-90.9	365^{a}	220-606	2.74^{b}	2.04-3.67
4	35.5^{a}	16.8-74.9	367^{a}	221-609	2.71^{b}	2.02-3.63

[1] Slope function = .5(LC 84/LC 50 + LC 50/LC 16)

[2] Values with same letter superscripts (between experiments) do not differ significantly ($P > .05$).

estimate for Experiment 2 was significantly lower than those obtained for other experiments, due to higher mortality at 100 ppb in Experiment 2. Overall, results conservatively estimate the 21 day LC 1 and LC 50 at 1 and 100 ppb atrazine, respectively.

Adenine Nucleotides--Adenylate and EC values, in replicate Experiments 1-4, are presented in Tables 7.51-7.54, respectively. These data were pooled, and mean values are displayed in Figure 7.26. In this figure, each experiment was weighted equally and adenylates at 1000 ppb atrazine were excluded, since data at this concentration were obtained in Experiment 1 only.

In this pooled analysis, EC was reduced at 0.1, 1.0, and 10 ppb atrazine over 21 days, but higher ATP at 100 ppb elevated EC to the control level. ADP and AT generally increased with higher atrazine levels. These observations were reflected in relatively strong and positive correlation of ATP, ADP, and AT with atrazine (Table 7.55).

Discussion

Short-Term (6 Hr) Atrazine Experiments--

Productivity--*Z. marina* net productivity was inhibited at 100, but not 10 ppb atrazine, over 6 hrs. Net productivity of the *Z. marina* community, isolated under large plexiglass domes in the field, was similarly depressed at 100 ppb atrazine during daylight hrs (Section V). Using laboratory microcosms, Correll et al. (1978) have reported a reduction of net productivity with 100 ppb atrazine in another submerged aquatic macrophyte, *Zanichellia palustris* (horned pondweed), after 1 and 2 week exposures.

Depression of oxygen evolution is expected, since atrazine inhibits the Hill reaction in photosynthesis (Ebert and Dumford, 1976). Although internal cycling of gases within lacunar spaces of leaves may have introduced error into production measurements, based on changes in dissolved oxygen (McRoy and McMillan, 1977), both control and test measurements should have contained the same error.

Adenine nucleotides--Adenylate levels in *Z. marina* decreased at both 10 and 100 ppb atrazine over 6 hrs. Since ATP, ADP, and AMP were reduced proportionately, EC ratios remained constant. Apparently, EC was stabilized by removal of AMP with adenylate deaminase (Chapman and Atkinson, 1973). It appears that ATP or AT serves as a more sensitive index of short-term herbicide stress than EC in *Z. marina*.

Z. marina adenylates, but not net productivity, were reduced at 10 ppb atrazine. This indicates that adenylate determinations were a more sensitive monitor of short-term herbicide stress than net productivity measurements. Noncyclic photophosphorylation may have been impaired with lower amounts of atrazine than photosynthetic oxygen evolution.

Long-Term (21 Day) Atrazine Experiments--

Morphometrics--Growth of *Z. marina*, as measured by shoot length and number of leaves, was clearly inhibited at 100 ppb atrazine over 21 days. It

TABLE 7.51. ADENINE NUCLEOTIDES (μG ATP EQUIV G^{-1} DRY WT) AND EC AFTER 21 DAY ATRAZINE EXPOSURE IN EXPERIMENT 1 (N=6)

Variable	Nominal Atrazine (ppb)					
	Control	.1	1.0	10	100	1000
ATP	$135^{a1} \pm 5^{2}$	$92^{b} \pm 9$	$160^{c} \pm 2$	$170^{d} \pm 1$	$220^{e} \pm 6$	$219^{e} \pm 5$
ADP	$56^{a} \pm 4$	$64^{a} \pm 4$	$106^{bc} \pm 3$	$119^{b} \pm 5$	$94^{c} \pm 3$	$106^{bc} \pm 5$
AMP	$86^{a} \pm 3$	$106^{b} \pm 3$	$188^{c} \pm 4$	$178^{c} \pm 7$	$150^{c} \pm 10$	$161^{c} \pm 23$
AT	$277^{a} \pm 11$	$262^{a} \pm 15$	$454^{b} \pm 6$	$467^{b} \pm 8$	$465^{b} \pm 20$	$487^{b} \pm 26$
EC	$.59^{a} \pm <.01$	$.47^{b} \pm .01$	$.47^{b} \pm .01$	$.49^{b} \pm .01$	$.58^{a} \pm .01$	$.57^{a} \pm .03$

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).
[2] Standard error.

TABLE 7.52. ADENINE NUCLEOTIDES (μG ATP EQUIV G^{-1} DRY WT) AND EC AFTER 21 DAY ATRAZINE EXPOSURE IN EXPERIMENT 2 (N=4)

Variable	Nominal Atrazine (ppb)				
	Control	.1	1.0	10	100
ATP	$141^{a1} \pm 6^{2}$	$90^{b} \pm <1$	$101^{c} \pm 5$	$78^{b} \pm 8$	$166^{d} \pm 5$
ADP	$83^{a} \pm 5$	$74^{a} \pm 3$	$75^{a} \pm 6$	$68^{a} \pm 5$	$108^{b} \pm 10$
AMP	$90^{a} \pm 3$	$131^{b} \pm <1$	$105^{a} \pm 4$	$89^{a} \pm 9$	$76^{a} \pm 8$
AT	$314^{a} \pm 6$	$294^{ab} \pm 4$	$280^{b} \pm 10$	$235^{c} \pm 9$	$350^{d} \pm 7$
EC	$.58^{a} \pm .01$	$.43^{b} \pm <.01$	$.50^{b} \pm .01$	$.48^{b} \pm .03$	$.63^{a} \pm .01$

[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

TABLE 7.53. ADENINE NUCLEOTIDES (μG ATP EQUIV G^{-1} DRY WT) AND EC AFTER 21 DAY ATRAZINE EXPOSURE IN EXPERIMENT 3 (N=6)

Variable	Nominal Atrazine (ppb)				
	Control	.1	1.0	10	100
ATP	98^{a}[1] ± 2[2]	111^{ab} ± 4	106^{ab} ± 3	110^{ab} ± 2	121^{b} ± 8
ADP	45^{a} ± 3	43^{a} ± 2	52^{a} ± 5	49^{a} ± 1	63^{b} ± 3
AMP	33^{a} ± 1	34^{a} ± 1	42^{ab} ± 5	45^{b} ± 2	47^{b} ± 4
AT	175^{a} ± 3	187^{a} ± 7	199^{b} ± 12	204^{b} ± 3	231^{c} ± 11
EC	$.69^{a}$ ± .01	$.71^{b}$ ± <.01	$.66^{a}$ ± .02	$.66^{a}$ ± .01	$.66^{a}$ ± .01

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[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

TABLE 7.54. ADENINE NUCLEOTIDES (μG ATP EQUIV G^{-1} DRY WT) AND EC AFTER 21 DAY ATRAZINE EXPOSURE IN EXPERIMENT 4 (N=6).

Variable	Nominal Atrazine (ppb)				
	Control	.1	1.0	10	100
ATP	164^{a}[1] ± 4[2]	164^{a} ± 10	157^{a} ± 4	169^{a} ± 5	202^{b} ± 2
ADP	60^{a} ± 4	78^{b} ± 4	76^{b} ± 3	87^{bc} ± 2	93^{c} ± 3
AMP	26^{a} ± 3	40^{bc} ± 3	51^{d} ± 4	48^{cd} ± 2	37^{b} ± 4
AT	250^{a} ± 7	282^{b} ± 12	284^{b} ± 3	304^{b} ± 7	332^{c} ± 6
EC	$.78^{a}$ ± .01	$.72^{b}$ ± .01	$.69^{b}$ ± .01	$.70^{b}$ ± <.01	$.75^{c}$ ± .01

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[1] Values with same letter superscripts (between treatments) do not differ significantly ($P > .05$).

[2] Standard error.

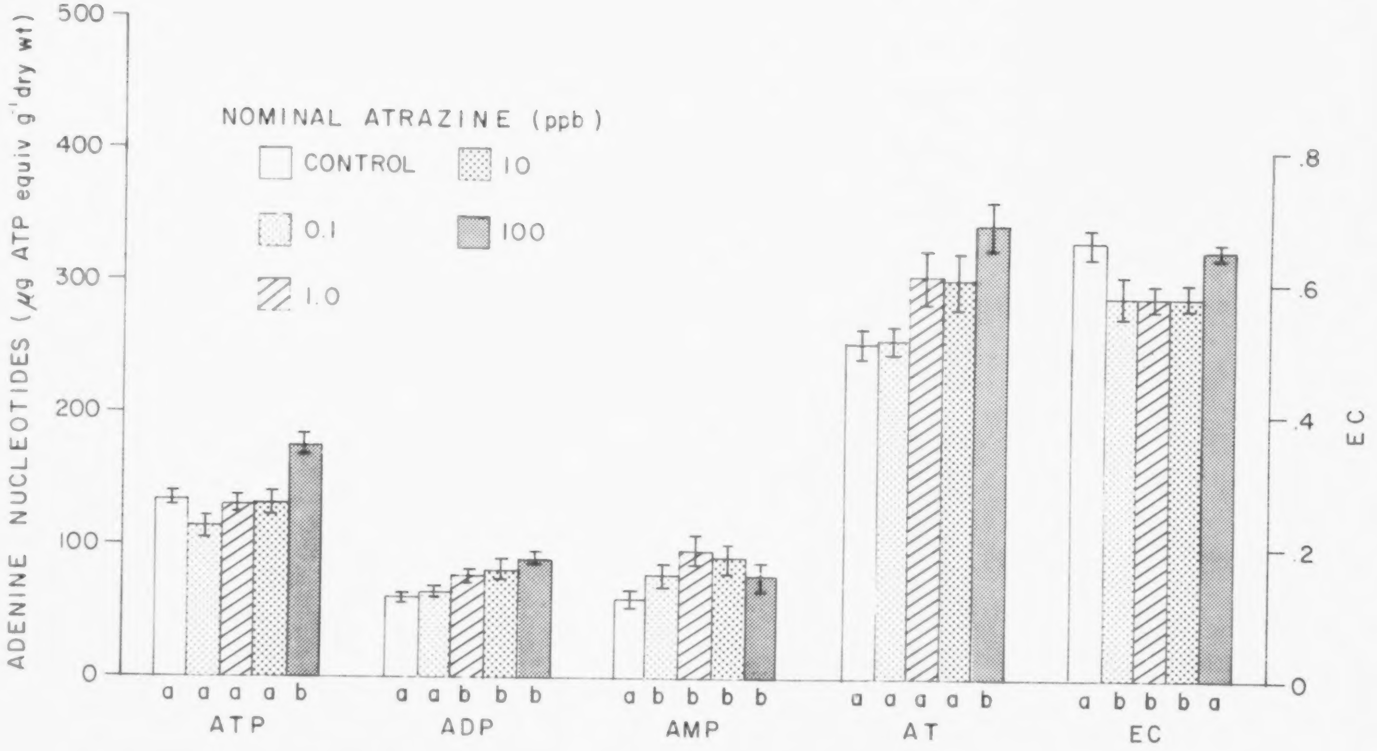

Figure 7.26. Adenine nucleotides and EC after 21 days in the long-term atrazine experiments. Data from replicate experiments are pooled (n=24). Values with the same letter do not differ significantly ($P>0.05$) among treatments. Error bars are 1 standard error.

TABLE 7.55. SPEARMAN CORRELATION COEFFICIENTS OF 21 DAY ADENINE NUCLEOTIDES AND EC WITH NOMINAL ATRAZINE CONCENTRATION IN LONG-TERM ATRAZINE EXPERIMENTS. MEANS FROM REPLICATE EXPERIMENTS ARE POOLED (N=20)

Variable	Correlation Coefficient
ATP	.3956
ADP	.4844*
AMP	.1901
AT	.3679
EC	-.0400

* P < .05

appears that 10 ppb was also inhibitory, but to a lesser extent. Apparent stimulation of *Z. marina* growth at 1 ppb may have been an indirect result of selective atrazine toxicity toward epiphytic algae, since epiphytes can inhibit *Z. marina* photosynthesis by interfering with carbon uptake and by reducing light intensity (Sand-Jensen, 1977). Other studies have demonstrated inhibitory effects of atrazine on algal photosynthesis (Plumley and Davis, 1980) and growth (Veber et al., 1981), although at higher concentrations.

Section VI reports 21 day EC 50 values (equivalent to I_{50}, the concentration inhibiting growth 50%) of 410 and 60 ppb atrazine for shoot length and number of leaves, respectively, with *Z. marina* in laboratory bioassays. Forney and Davis (1981) have calculated 3-6 week I_{50} values of 80-1104 ppb atrazine, based on leaf length measurements with other submerged aquatic macrophyte species under various laboratory conditions. These results are in general agreement with those of the present study.

Mortality--Conservative estimates of the 21 day LC 1 and LC 50 (concentrations lethal to 1 and 50% of the test organisms, respectiively) for *Z. marina* are 1 and 100 ppb atrazine, respectively. Forney and Davis (1981) have calculated LC 1 and LC 50 values of 11 and 53 ppb atrazine, respectively, for *Potamogeton perfoliatus* (redheadgrass pondweed).

Tolerance of plants toward triazine herbicides may have been influenced by temperature (Ebert and Dumford, 1976). Incomplete mortality at 1000 ppb atrazine, over 21 days in replicate Experiment 1, may be related to cooler prevailing temperatures. Atrazine toxicity appears to increase with warmer temperature, perhaps due to accelerated rates of uptake and translocation.

Adenine nucleotides--Inability to remove AMP from the adenylate pool contributed to a reduction in *Z. marina* EC at 0.1, 1.0, and 10 ppb atrazine over 21 days. At 100 ppb, corresponding to the estimated LC 50, ATP and EC unexpectedly rebounded before plant death resulted. Apparently, severe stress (100 ppb) elicits an adaptation response. For example, increased rates of respiration and associated oxidative phosphorylation may have supplied ATP in sufficient amounts to maintain metabolic homeostasis. Continued stress at 100 ppb atrazine, however, became lethal.

ATP and AT response patterns at 100 ppb atrazine appear to follow the triphasic general adaptation syndrome, outlined by Selye (1976). Over the short-term (6 hrs), ATP and AT were reduced (alarm reaction). Over the long-term (21 days), ATP and AT increased beyond control levels (stage of resistance) until death resulted (stage of exhaustion). Giesy et al. (1981) have reported a similar response pattern for ATP, AT, as well as EC, in *Palaeomonetes paludosis* (glass shrimp) with 30 g l^{-1} cadmium exposure.

Morphometric and mortality data facilitate interpretation of adenylate response. EC indicated stress as low as 0.1 ppb atrazine, but failed to reflect visually apparent stress at 100 ppb. It appears, then, that EC is a sensitive monitor of long-term, sublethal herbicide stress. When *Z. marina* was confronted with more severe stress, however, physiological adaptation increased EC before death resulted. The utility of EC as an index of long-term herbicide stress in *Z. marina* may, therefore, be limited.

Conclusions--

Adenylate and EC response in Z. marina to selected environmental variables are useful measures of metabolic state under certain conditions. The response is integrative, representing the interaction of genetic disposition with the environmental matrix, both stressful and beneficial. This may be advantageous in an ecological context, but can pose difficulties when attempting to evaluate effects of a single variable. Adenylate and EC response may also change in accordance with physiological adaptation over time. Chronic and severe herbicide stress was observed to elicit this adaptive response in Z. marina.

ATP or AT response may be more appropriate than EC in certain cases, as a monitor of environmental stress. ATP and AT decreased in Z. marina with short-term herbicide stress, but EC remained constant. In contrast, EC was reduced with long-term, sublethal herbicide stress. Limitations of adenylate and EC utility must be recognized in order to allow sound interpretation of results. It is suggested that more conventional quantitative analyses accompany adenine nucleotide measurements in any effort to evaluate physiological response to environmental variation.

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